WATER PERMEABILITY OF ISOLATED TISSUES FROM THREE SPECIES OF DECAPOD CRUSTACEANS WITH RESPECT TO OSMOTIC CONDITIONS AND EFFECTS OF NEUROENDOCRINE FACTORS

by

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ABSTRACT

WATER PERMEABILITY OF ISOLATED TISSUES FROM THREE SPECIES OF DECAPOD CRUSTACEANS WITH RESPECT TO OSMOTIC CONDITIONS AND EFFECTS OF NEUROENDOCRINE FACTORS

by

ANGELA CRISTINI CANTELMO

Advisor: Dr. Linda Mantel

The diffusional permeability to water of the gill, gut, and muscle tissues of three species of decapod crustaceans, (Cancer irroratus, Callinectes sapidus, and Libinia emarginata) was measured using THO (tritiated water). C. irroratus and C. sapidus were acclimated to 100% and 40% sea water, L. emarginata were maintained in 100% sea water. Results were expressed as % saturation values, that is the amount of THO found on the hemolymph side of the tissue, compared to the initial amount of THO in the outside medium, which was considered to be 100%.

Gill and gut tissues of C. irroratus and C. sapidus acclimated to 40% sea water have per cent saturation values that are significantly lower than those of the same species acclimated to 100% sea water. Thus, C. irroratus and C. sapidus can reduce the permeability of their tissues to water on acclimation to a lower salinity. Within each acclimation group, there are no significant differences in THO

influxes between the gill and gut tissues exposed to water at the salinity of acclimation and the gill and gut tissues exposed to water at 10% of the acclimation salinity. However, the THO uptake of the muscle tissue of all three species of decapods subjected to a stress of 10% of the salinity of acclimation is significantly higher than the THO uptake of the muscle tissue not subjected to a stress. The THO influx across the gill and gut tissues of L. emarginata and C. sapidus is reduced in the winter, which corresponds to the seasonal reduction in general activity of both species.

The diffusional permeability to water was measured for the gill and gut tissues of C. sapidus and L. emarginata in the presence and absence of neurohormonal factors. Neuroendocrine extracts from the thoracic ganglionic mass and the eyestalks reduce the permeability to water of gill and gut tissues of both species. Libinia emarginata and Callinectes sapidus acclimated to 100% sea water have hormonal factors present in their neuroendocrine tissues that reduce the THO influxes of the epithelial tissues such as the gill and gut. The extracts of neuroendocrine tissues from C. sapidus acclimated to 40% sea water appear to effect the greatest reduction in permeability to water. The neuroendocrine factors are not species specific in their action. The gill tissue exhibits a greater response to the neurohormonal factors than does the gut tissue.

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INTRODUCTION

One of the most important relationships between a marine or estuarine organism and its environment concerns the organism's hydromineral balance. Estuarine organisms that enter water of low salinities must actively regulate their content of salt and water in order to avoid fatal dilution of their tissues. Additionally, in crustaceans, at the time of ecdysis, the regulation of salt and water balance becomes particularly important because of the need to take up water for successful molting and growth. Precise hydromineral regulation necessary for ionic homeostasis and molting has been shown to be under hormonal control (Bliss et al, 1966; Mantel, 1968; Kamemoto and Tullis, 1972; Fingerman and Heit, 1975). However, many questions regarding hydromineral regulation in decapod crustaceans and the effect of neurohormones thereon remain unanswered.

Decapod crustaceans inhabit many environments and exhibit a wide variety of osmoregulatory capabilities. Stenohaline forms that are usually restricted to a marine environment have no osmoregulatory capacity and are osmoconformers. The more euryhaline forms inhabit the estuaries and are capable of varying degrees of esmoregulation.

There is a great deal of descriptive material on the osmoregulatory ability of many different species of crustaceans. However, less information is available concerning the mechanisms of osmoregulation. Studies establishing the osmoregulatory abilities of estuarine decapods have been reviewed by Potts and Parry (1964),

Lockwood (1968), Schoffeniels and Gilles (1970) and Kinne (1971). Many workers have shown that estuarine decapods subjected to a hyposmotic stress are able to withstand dilution and are able to maintain the concentration of ions in the hemolymph above that of their environment (Mantel, 1967; Gross, 1964; Lockwood, 1965; Cantelmo et al, 1975). In order to accomplish this, the organism must actively take up ions from the medium. The active uptake of ions was first demonstrated by Nagel (1934). His classic experiment showed conclusively that the euryhaline crab Carcinus maenas was able to take up Cl^- against a concentration gradient. Krogh (1938) showed that the decapod Eriocheir sinensus could take up Na^+ and Cl^- independently from dilute media.

Since the active uptake of ions was first demonstrated, there has been considerable research dealing with the sites and rates of transport. The gills of crustaceans have long been thought to be the primary sites for active transport. Croghan (1958) found that the gills of the brine shrimp, Artemia salina, stained with $AgNO_3$; this suggested that a high concentration of Cl^- was present. He also found that when the gill tissue was destroyed the animals could no longer osmoregulate. Mantel (1967) studied the radioactive ion fluxes and potential differences across the gills of Callinectes sapidus. She demonstrated, by means of potential differences, that active transport was taking place across the gills. She also showed that the rates of flux of Na^+ and Cl^- varied with the salinity to which the animals were acclimated. Histological studies such as the one performed by Copeland (1964) indicate that the gill tissues of osmoregulating crabs are the

type that are usually associated with active transport. The cells of the gill lamellae have numerous mitochondria, a large amount of rough endoplasmic reticulim; they also possess more highly organized microtubules than do somatic cells.

The ATPase enzyme system has been suggested as the enzymatic basis for Na^+ and K^+ transport in crustacean tissues. Quinn and Lane (1966) have indicated that a Na^+ and K^+ stimulated system in gill homogenates of the land crab Cardisoma guanhumi participates in ionic regulation. Both Mantel (1976) and Towle (1976) have demonstrated that the Na^+, K^+, activated ATPasa activity in the gill tissues of Callinectes sapidus is much higher when the crabs are acclimated to a low salinity then when they are in full sea water. This indicates a greater amount of active transport in these tissues when the animals are osmoregulating.

There have been many studies that suggest that the crustacean gut is also an ionically active tissue. Dall (1965, 1967) has studied the gut of shrimp and crabs with respect to ion regulation. Heeg and Cannone (1966) found that the hind gut of two species of crabs is involved in osmoregulation. Mantel (1968) found that the forgut of Gecarcinus lateralis is an osmotically active tissue that is permeable to both water and salts. Thus it seems that tissues forming the boundaries between the organism and its environment are the osmotically active tissues in an animal.

Another important mechanism to prevent excess loss of salts and uptake of water as a result of estuarine penetration is to reduce the integumental permeability to salts and water. This has been well documented in the case of salts by Nagel (1934) and Gross (1957).

They showed that marine crabs are much more permeable to salts than are euryhaline estuarine decapods. Rudy (1967), Smith (1967, 1970), Smith and Rudy (1972), Capen (1972), and Hannen and Evans (1973) studied the decrease in diffusional permeability to water in crustaceans exposed to dilute salinities. There have been some conflicting results, perhaps because of a small isotope effect between two different tracers and different experimental temperatures (Smith and Rudy, 1972). However, it has been demonstrated that a variety of euryhaline crustaceans (i.e. Carcinus meanus, Rhithropanopeus harrisii, Hemigrapus nudus, and Limulus polyphemus) are capable of decreasing their "apparent permeability" to water upon exposure to a hyposmotic medium. The experiments consisted of placing the whole animal into a large bath containing radioactively labeled water for a period of time and measuring the radioactivity in samples of hemolymph. The reduced exchange of water that was observed in these experiments could have resulted from a reduction in epithelial permeability to water. However, reduction in circulation of blood to the gills or reduction in irrigation of the gills could also result in less radioactive label entering the hemolymph. Thus, the term "apparent permeability" was used (Smith, 1967). In these studies, diffusional fluxes of water were measured in whole animals, but permeability of the different tissues during the salinity stress was not examined. As mentioned ablve, the main sites of ionic activity associated with hyperosmoregulation are the epithelial tissues such as the gill and gut. It is also likely that these tissues are involved in the movement of water and ions at all times and in all environments. Thus examination of the perme-

ability to water of these tissues should add to the understanding of the mechanisms necessary for survival in a marine or estuarine environment.

Intracellular homeostasis requires a constant ionic composition within cells. Cellular volume must be regulated in order to prevent lysis on exposure to a reduced salinity. Therefore, ionic and osmotic regulation is necessary and important at the cellular level. The adaptation of cells to changes in osmotic concentrations has been termed intracellular isosmotic regulation. Lang and Gainer, (1969), Gilles (1972), Gerard and Gilles (1972), and Haberfield and Hass (1975) have all examined the volume changes in isolated muscle and nerve fibers of different euryhaline crustaceans subjected to a salinity stress. All of the tissues showed an initial increase in volume, then later, after a period of time, returned toward the initial volume. Studies have shown that amino acid pools play an important role in regulation of volume and osmotic equilibrium between the cells and surrounding fluid in euryhaline regulators. Gerard and Gilles (1972) and Gerard (1975) have demonstrated that excretion of free amino acids from cells is an active process, which decreases the cellular osmotic pressure and allows osmotic equlibrium to be maintained. Most of these studies have examined the amino acid concentration of various tissues or the volume changes of nerve and muscle fibers. To date there have been no studies that have examined the permeability to water of both the epithelial and internal tissues.

All of the studies discussed above reveal a fairly comprehensive description and some understanding of the processes involved in

hydromineral regulation. However, they do not take into account possible neuroendocrine controls of salt and water balance.

As early as 1947, Scudamore reported that removal of eyestalks or sinus glands from crayfish resulted in an increase in water content of the animals; these changes could be corrected by implantation of the sinus gland. Since that time, many workers have studied the neuroendocrine control of salt and water balance in decapod crustaceans including Carlisle (1955); Bliss et al. (1966); Kamemoto et al. (1966); Skinner et al. (1965); Kamemoto and Tullis (1972); Tullis and Kamemoto (1973); Mantel (1968); Tullis (1974); and Mantel et al. (1975). Kamemoto and Tullis (1972) studied the neuroendocrine controls of salt and water regulation in intermolt specimens of fresh water crayfish. They injected fractionated extracts of the brain and thoracic ganglionic mass (TGM) into normal and eyestalkless animals. The data indicated that one fraction increased the concentration of Cl^- in the hemolymph and the flux of Na^+ from the medium. However, some results could not be repeated, perhaps because of seasonal cycles. Other workers have also suggested that hormonal factors control water and ion movements in decapod crustaceans. Rammamurthi and Scheer (1967) noted that the cephalothorax extract from the prawn Pandalus jordani decreased the efflux of Na^+ from the crab Hemigrapus nudus. Tullis and Kamemoto (1974), in one of their most recent papers, present evidence that there are at least two factors that can be separated from extracts of the brain and thoracic gangionic mass of Thalamita crenata. A water soluble fraction causes a decrease in influx of tritiated water, while an acrtone soluble fraction causes an increase

in influx of tritiated water when injected into a crab or a crayfish. Neither fraction affects sodium influx. Bliss et al. (1966) have given evidence suggesting that there are at least two hormones in Gecarcinus lateralis that control retention and removal of water.

Both Kamemoto (1976) and Kleinholz (1976), in their review papers on neuroendocrines in crustaceans, point out that a variety of experimental evidence suggests the involvement of the eyestalk system, the brain, thoracic ganglionic mass, and the pericardial organs in neuroendocrine control of hydromineral balance. There are many conflicting data on the effects of removal of eyestalks and the injection of various extracts into whole or eyestalkless animals. For example, Kamemoto (1976) points out that injection of extracts of eyestalks, TGM, and brain have caused an increase in salt and osmotic concentration of the hemolymph in some animals and a decrease in salt and osmotic concentration of the hemolymph in other animals. Most of the work in the literature has been performed on whole animals or in the fom of bioassay experiments, where the neuroendocrine extracts of one species are injected into another species. In some cases the two species come from different environments and are subject to widely different osmoregulatory demands. When extracts are injected there is no way of knowing how these extracts interact with the neurohormones that are already present in the animal, and what effect this interaction might have on the tissues.

Few studies have examined the effects of neurohormones on isolated tissues. Mantel (1968) examined the isolated forgut of G. lateralis in vitro and Tullis (1974) studied the gills of T. crenata

in vivo. The results of these and the previous studies suggest the need for in vitro studies to establish the effects of neuroendocrine factors on the water permeability of isolated epithelial tissues, such as the gill and gut.

Lockwood and Inman (1973); Lockwood and Andrews (1969) and Dandifosse (1966) have studied changes in water and ion permeability of different crustaceans at ecdysis. All the studies indicate that increases in exchange of water and ions occur and are necessary for successful ecdysis. Removal of eyestalks from decapods such as G. lateralis, C. immunis, and C. meanas is known to produce large increases in size due to excessive water uptake at ecdysis (Abramowitz and Abramowitz, 1940; Scudamore, 1947; Carisle, 1955). Working with G. lateralis, Bliss et al. (1966) and Mantel (1968) suggest that the control of water permeability if by means of neuroendocrine factors and is probably important in the conservation of water, especially at ecdysis. Further research on the effects of neuroendocrine substances on tissue permeability might help to explain the neurohormonal control function in ecdysial water balance.

This brief survey of the literature in crustacean osmoregulation and neurohormonal control of hydromineral balance reveals certain areas where further study is necessary. Research concerning the water permeability of the ionically active tissues such as the gill and gut, and other more internal tissues such as muscle, in crabs with different osmoregulatory abilities under different salinity conditions is necessary. Such studies would yield substantial information that would help in the understanding of the mechanisns and processes of

osmoregulation. A study coupling observations on water permeability of isolated tissues with observations on the effects of neuroendocrine extracts on permeability of these tissues would help to clarify the controls of water movement. Therefore, I have designed a study to examine the diffusional permeability to water of isolated tissues from dacapods subjected to different salinity conditions. Studies have also been designed to examine the water permeability of tissues in the presence and absence of added neuroendocrine extracts.

The decapods chosen for study are: Cancer irroratus, Say, Callinectes sapidus, Rathbun, and Libinia emarginata, Leach. C. irroratus, the common rock crab, and C. sapidus, the blue crab, are abundant, easily collected euryhaline decapods that inhabit Atlantic coastal estuaries. The portunid crab C. sapidus was selected because of its well documented osmoregulatory ability (Gifford, 1962; Tan and Van Engèl, 1967; Mantel, 1967; Tagatz, 1971; Lynch et al., 1973). The cancroid crab C. irroratus has been shown to be an osmoregulator, but it appears to have a more limited estuarine distrubution than C. sapidus (Cantelmo et al., 1975). Libinia emarginata, the brachyuran spider crab, is a decapod that inhabits the Atlantic Ocean. Gilles (1970) has demonstrated that L. emarginata is an osmoconforming crab that is not able to regulate the concentration of its hemolymph above that of the external environment when exposed to a hyposmotic medium. Studies on the water permeability of the gill, gut, and muscle tissues were carried out on the three species of decapods. Experiments on the effects of the neurohormonal extracts were carried out on L. emarginata and on C. sapidus.

MATERIALS AND METHODS

COLLECTION AND MAINTAINANCE OF ANIMALS

At all seasons, specimens of *Cancer irroratus* and *Callinectes sapidus* were collected at Sandy Hook Bay, New Jersey from a depth of 10-30 meters with an otter trawl and/or crab rake. During the winter months, specimens of *C. sapidus* were also purchased from Randazo's Fish Store, Bronx, New York. In the summer season, individuals of *Libinia emarginata* were collected by commercial fish trawlers, who trapped the animals in the Atlantic Ocean off the coast of New Jersey. During the winter, specimens of this species were purchased from the Gulf Specimen Company, Panacea, Florida.

The experiments performed during the course of this study were carried out during two seasons of the year. The work on the diffusion of tritiated water (THO) in the presence or absence of an osmotic gradient was performed with animals collected in July and August 1975. The experiments examining the effects of extracts of neuroendocrine tissue were performed on animals collected from January through May 1976.

All animals were maintained in individual 4-6 liter plastic containers inside an environmental chamber. The temperature inside the chamber was 15°C, and a 12L: 12D illumination cycle was maintained. All specimens of *L. emarginata* and one half of the specimens of *C. sapidus* and *C. irroratus* were maintained in 100%

(33 ppt, 1000 milliosmols) Instant Ocean Sea Water. The remaining individuals of C. irroratus and C. sapidus were gradually acclimated to 40% (13 ppt, 400 milliosmols) Instant Ocean Sea Water. Water in the containers were aerated at all times. The animals were fed fresh clams once per week, and the water was changed after feeding. The crabs were allowed two weeks to adjust to the experimental conditions before the experimentation began.

The stage of the intermolt cycle was determined according to the criteria of Drach (1939). After the animal was sacrificed, a section of the carapace was removed and examined for the presence or absence of the membranous layer. If the membranous layer was not found, the animal was discarded. This insured that all animals used for experimentation were in the C-4 stage of their cycle. All experiments were performed at 25°C.

EXPERIMENTAL PROCEDURES

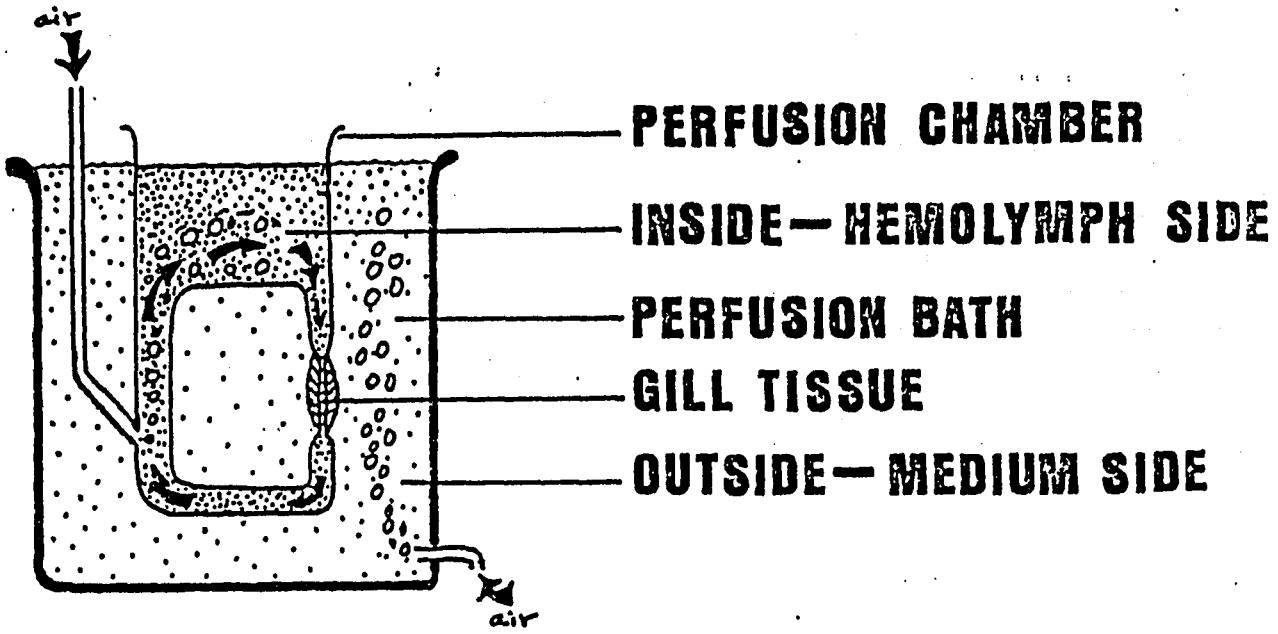
Before the animals were sacrificed, a sample of the hemolymph and medium was taken, and the osmolality of each was measured with an Advanced Instruments Osmometer. Crabs were then sacrificed by destroying the brain with a needle inserted through the eye socket. The gill and gut tissues were removed and mounted on perfusion chambers modified from Mantel (1967). In some experiments, the muscle tissue was also dissected from the animal and pieces were placed in individual Erlenmeyer flasks.

Individual gills were removed from the branchial chamber, the tip of the gill was cut off, and the clotted hemolymph was removed

from the afferent artery. The gill was then ligated onto the open ends of the perfusion chamber with cotton thread, thus forming a continuously circulating system (Fig. 1A). Circulation of the fluid is accomplished by bubbling air from a pump. When this air lift system is activated, the fluid in the perfusion chamber is forced to flow through the efferent artery and the gill lamellae. This flow was verified by the use of a solution of methylene blue dye. The flow rates within the perfusion chamber were calibrated by measuring the flow of glass microbeads, and the rates remained constant through out the experiments. The fluid in the perfusion bath simulated the fluid on the outside or medium side of the gill; while the fluid in the perfusion chamber simulates the fluid on the inside or hemolymph side of the gill. The perfusion bath has a volume of 50 ml and the perfusion chamber has a volume of 5 ml. After termination of the experiment, the gills were cut away from the chambers and weighed on a Mettler balance to 0.1 mg.

The gut tissue was dissected from the crab, and a piece was mounted on the glass perfusion chamber by means of a rubber "O" ring (Fig. 1B). The fluid in the perfusion bath simulates the fluid on the hemolymph side of the gut; while the fluid in the perfusion chamber simulates the fluid on the lumen side of the gut. The perfusion bath has a volume of 40 ml and the perfusion chamber has a volume of 5 ml.

Tritiated water (THO) was added to the perfusion bath of the gill preparation (0.5 μCi/ml) and to the perfusion chamber of the gut preparation 5.0 μCi/ml). At specified intervals, by means of a 100 λ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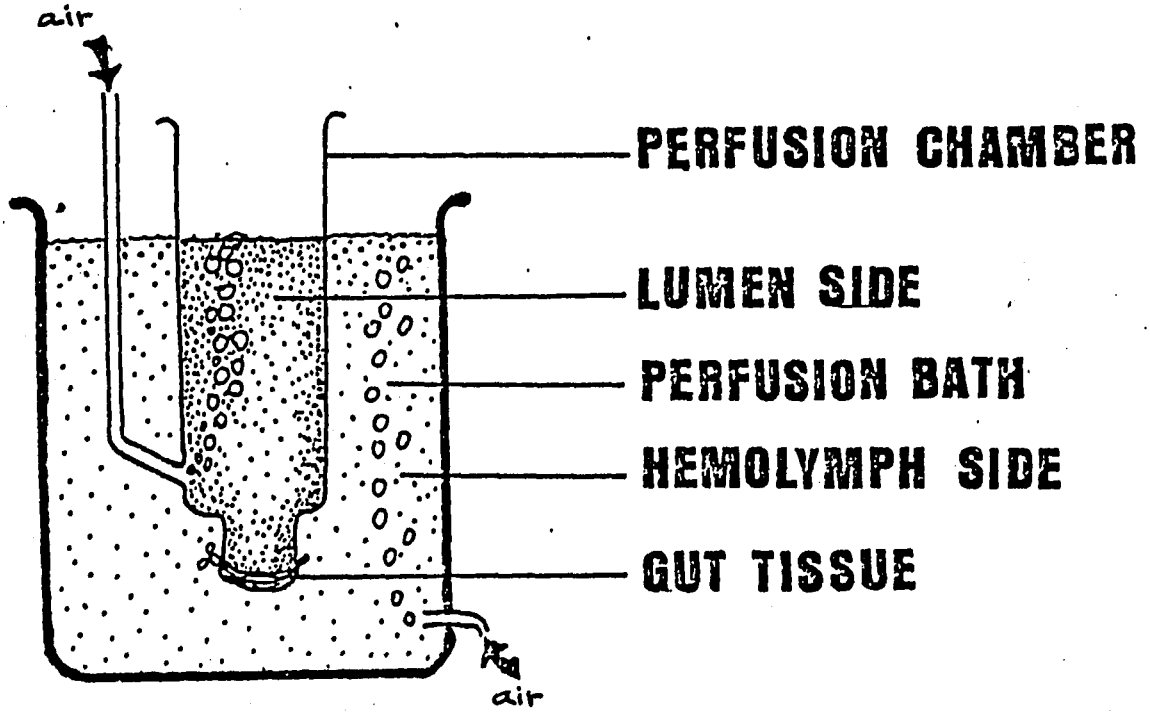
INSIDE—HEMOLYMPH SIDE
PERFUSION BATH
GILL TISSUE
OUTSIDE—MEDIUM SIDE
air

A


air
PERFUSION CHAMBER
LUMEN SIDE
PERFUSION BATH
HEMOLYMPH SIDE
GUT TISSUE
air

B

FIGURE 1

Oxford Micropipettor, samples were drawn from the perfusion chamber of the gill preparation and from the perfusion bath of the gut preparation. In the same manner, before the tissues were added, samples were taken that served as standards. All of the samples were counted as described below. Thus, this set of experiments measured the diffusion of THO from the medium to the hemolymph side of the gill and from the lumen to the hemolymph side of the gut.

Pieces of muscle tissue were removed from the proximal segment of the chela and walking legs of the animals. These pieces were cut into smaller pieces, and the smaller pieces incubated in individual aerated 25 ml glass Erlenmyer flasks. Tritiated water (1.0 μCi/ml) was added to each of these incubation flasks. A sample that served as a standard was taken with a 100 λ Oxford Micropipettor before the tissue was added. Tissues were removed at specified intervals, rinsed in unlabeled medium, placed in pre-weighed Oximat acetate cups, and weighed to 0.1 mg. The cups and the tissues were subsequently combusted in an Oximat Tissue Oxidizer. This allowed the THO that was contained in the tissues to be released. These experiments measured the diffusion of THO from the outside of the tissue into both the intracelluar and extracellular spaces of the crab muscle.

All liquid samples were added to 15 ml of Instagel and counted in a Beckman model LS - 250 scintillation counter. The quench was determined to be negligible. The THO that was contained in the muscle tissue was released into the Oxifluor Scintillation Fluid inside the Oximat machine. The liquid samples that came from this machine were counted on a Beckman counter, and corrections were made for carry-over and background.

EXPERIMENTS ON DIFFUSION OF THO IN THE PRESENCE AND ABSENCE OF AN OSMOTIC GRADIENT

This set of experiments was designed to show the effects of acclimation and osmotic stress on the diffusion of THO across the epithelium of the gill and gut and into the intracellular and extracellular spaces of the muscle tissue.

For each individual of C. sapidus, C. irroratus and L. emarginata that was tested in this set of experiments, one-half of the samples of the gill, gut and muscle tissue was exposed to a medium with an osmolality equivalent to the salinity of acclimation; this salinity was 100% or 40% and is designated the control salinity. The other half of the samples were exposed to an osmotic stress consisting of 10% of the control salinity.

Since four gills of each animal were used, 2 gills were perfused on the medium side with the control salinity, while the other two were perfused on the medium side with the stress salinity. The hemolymph side of the gills was always perfused with sea water of the control salinity. Each gut was divided into two pieces. One piece was perfused on the lumen side with water of the control salinity. The other piece was perfused on the lumen side with water of the stress salinity. The hemolymph side of the both pieces of the gut was always perfused with water of the control salinity.

The gill and gut tissues were perfused in the test salinities for two hours. Samples were taken from the perfusion chamber of the gill

apparatus at the end of each hour. Samples from the perfusion bath of the gut apparatus were taken at 30 minutes, 1 hour and 2 hours.

Sixteen pieces of muscle tissue were used. Eight pieces were placed in perfusion fluid (Prosser and Brown, 1961) with an osmolality corresponding to the osmolality of the hemolymph of the crab at the salinity of acclimation; this is designated control perfusion fluid. The other eight pieces were incubated in perfusion fluid with an osmolality consisting of 10% of the osmolality of the hemolymph; this is designated stress perfusion fluid (Table 1). Two pieces of the muscle tissue were removed from the control and the stress perfusion fluid after 10 minutes, 20 minutes, 30 minutes, and 45 minutes.

EXPERIMENTS USING EXTRACTS OF NEUROENDOCRINE TISSUES

This set of experiments was designed to examine the effects of extracts made from the thoracic ganglionic mass (TGM), eyestalks (ES), and nerve-muscle (NM) on the diffusion of THO from the medium to the hemolymph side of the gill and from the lumen to the hemolymph side of the gut.

Extracts of the ES, TGM, and NM were made according to the method Kamemoto and Tullis (1974). The ES, TGM, and legs of crabs were removed and stored in the freezer for no longer than two weeks before being used. Approximately one hour prior to the actual experiment, the tissue to be extracted was removed from the freezer. The TGM was used in toto, while the ES were cut open and the soft tissue was removed. The legs were snapped at the joint between the carpus and

TABLE 1

Acclimation and test osmolalities for the muscle tissue.

Species	Salinity of Acclimation (%S.W.)	Hemolymph Osmolality (mosmols)	Test Osmolality (mosmols)
C. irroratus	100%	1000	1000 control (c)
C. irroratus	100%	1000	100 stress (s)
C. irroratus	40%	500	500 c
C. irroratus	40%	500	50 s
C. sapidus	100%	1000	1000 c
C. sapidus	100%	1000	100 s
C. sapidus	40%	750	750 c
C. sapidus	40%	750	75 s
L. emarginata	100%	1000	1000 c
L. emarginata	100%	1000	100 s

the merus and the leg nerve and some muscle tissue was removed. The tissues were ground with a mortar and pestle in 1 ml of perfusion fluid, with autoclaved sea sand to aid in grinding. After the tissues were ground, the test tube containing the extract was suspended in a boiling water bath for ten minutes. The resulting suspension was then centrifuged at 10,500 R.P.M. for 30 minutes in a Sorvall model RC2(3) centrifuge at 4°C. The supernatant was stored on ice for up to two hours until it was used in the perfusion system.

Gill and gut tissues of C. sapidus and L. emarginata were used in this set of experiments. All gill and gut tissues had sea water of the control salinity on both sides of the perfusion system, so that there was no osmotic stress. For each animal, four gills and two pieces of gut were tested. Two gills and one piece of gut were treated as controls by exposing them only to sea water at the control salinity. The remaining two gills and piece of gut were considered as experimentals by treating them with control salinity sea water containing extracts of one of the above tissues (i.e. TGM, ES, or NM). All tissues were first mounted with non-radioactively labeled sea water in the perfusion bath and in the perfusion chamber. Then 0.3 ml of extract was added to the perfusion chamber of the gill apparatus and to the perfusion bath of the gut apparatus. The other two gills and piece of gut received no extracts. All of the tissues were perfused in the unlabeled baths, either with or without extracts, for 20 minutes. After that time, a perfusion bath containing THO was replaced into the gill apparatus and 5 ml of THO labeled sea water was substituted into the perfusion chamber of the gut apparatus. The

tissues were perfused in these solutions for one hour before samples were taken from the hemolymph side.

Dosage of the neuroendocrine extracts was as follows: Tissues of C. sapidus acclimated to 100% sea water were treated as described above with the following extracts; (A) 4 TGM or 8 ES from specimens of C. sapidus acclimated to 100% sea water. (B) 2 TGM or 4 ES from specimens of C. sapidus acclimated to 40% sea water. (C) NM extracts from 2 legs of C. sapidus acclimated to 100% sea water.

Tissues of C. sapidus acclimated to 40% sea water were treated as described above with the following types of extracts: (A) 4 TGM or 8 ES from specimens of C. sapidus acclimated to 40% sea water. (B) NM extracts from two legs of C. sapidus acclimated to 40% sea water.

Tissues of L. emarginata acclimated to 100% sea water were treated as described above with the following types of extracts. (A) 4 TGM or 8 ES from specimens of L. emarginata acclimated to 100% sea water. (B) 2 TGM or 4 ES from specimens of C. sapidus acclimated to 40% sea water. (C) NM extracts from two legs of L. emarginata acclimated to 100% sea water.

ANALYSIS OF DATA

All results are expressed as percent saturation values; that is, the amount of THO found on the hemolymph side of the tissue, compared to the initial amount of THO in the outside or the lumen side medium, which was considered to be 100% (Smith, 1970). The percent saturation values were expressed per gram of gill and muscle tissue and per cm^2

of gut tissue. The hourly water exchange fraction, or rate constant K, was calculated according to Smith (1970) by use of the following equation: $K = (2.3/t)\log_{10} (100/100-\%\ sat.)$, where K= the percent of body water exchanged per hour, t= time of exposure in hours, % sat.= concentration of THO on the hemolymph side, of the tissues compared to the concentration of THO on the outside or lumen side of the tissue. In all cases, the specific activity of the radioactively labeled medium was high in relation to that of the non-radioactively labeled medium. This it can be assumed that dilution by water from the unlabeled side is negligible. At t= infinity, the percent saturation will approach 100%. In the calculation of K in this paper, t= 1 hour was used for both gill and gut.

Statistical significance of the data was taken at the 5% level of confidence by the use of the analysis of variance and Student's t-test, when required. The results are presented as percent saturation values; however, in order to perform the analysis of variance, the date were transformed by the use of the arc-sine transformation. The reference for all statistical methods was Sokal and Roth (1973).

RESULTS

OSMOTIC RESPONSES

Results of the osmolality measurements are shown in Table 2. All three species are osmoconformers in 100% sea water, since there are no significant differences ($P>0.05$) between the external medium and the hemolymph of the crabs ($P>.05$) (Table 2, ln 1,3,5). In 40% sea water, both C. irroratus and C. sapidus are hyperegulating, since the osmolality of their hemolymph is significantly higher ($P<.05$) than that of the external medium (Table 2, ln 2,4). The values also indicate that C. sapidus is a stronger regulator than C. irroratus.

DIFFUSION OF THO IN THE PRESENCE OR ABSENCE OF AN OSMOTIC GRADIENT

The data from experiments on the THO influx across the gills and gut are presented in Tables 3 and 4. A summary of the significant differences is presented in Table 5A. The most important and significant differences are seen when the % saturation values of the tissues of crabs acclimated to 100% sea water are compared with the % saturation values of the tissues of crabs acclimated to 40% sea water (Table 3, ln 1,3,5,7; Table 4, ln 1,3,5,7). Gills and guts of C. sapidus and C. irroratus acclimated to 40% sea water have significantly lowere($P<.001$) THO influxes than the tissues of crabs of the same species acclimated to 100% sea water (Table 5A, ln 1&2, 4&5; Fig. 2,3). The % saturation values at 40% sea water are less than

TABLE 2

Osmoregulatory ability of three species of crabs. Comparison of the osmolality of the hemolymph and medium with the probability that the differences between them are significant.

	Species	Number of Animals	Mean Medium Conc. (milliosmols)	Mean Hemolymph Conc. (milliosmols)	P
1.	Cancer irroratus	7	1031.8 ± 10.0	1015.5 ± 14.2	>0.05
2.	Cancer irroratus	7	404.5 ± 4.8	486.8 ± 3.2	<0.05
3.	Callinectes sapidus	6	1010.1 ± 10.6	1019.1 ± 13.9	>0.05
4.	Callinectes sapidus	6	424.1 ± 4.8	747.8 ± 13.3	<0.05
5.	Libinia emarginata	8	1029.5 ± 12.9	1026.5 ± 11.6	>0.05

TABLE 3

Water permeability of the gill tissues of three species of crabs as indicated by the percent THO saturation at one hour and the hourly water exchange fraction (K).

	Species	Number of Animals	Salinity of Acclim. (‰S.W.)	Salinity Acute Treat. (‰S.W.)	Mean % Sat. (1hr.)	K
1.	C. irroratus	6	100	100	78.82 ± 16.55	1.55
2.	C. irroratus	6	100	10	91.00 ± 15.23	
3.	C. irroratus	6	40	40	30.12 ± 4.40	0.35
4.	C. irroratus	6	40	4	26.82 ± 4.28	
5.	C. sapidus	6	100	100	67.83 ± 16.35	1.13
6.	C. sapidus	6	100	10	63.25 ± 20.00	
7.	C. sapidus	6	40	40	28.39 ± 6.82	0.33
8.	C. sapidus	6	40	4	28.27 ± 5.96	
9.	L. emarginata	6	100	100	75.42 ± 11.53	1.39
10.	L. emarginata	6	100	10	60.07 ± 7.06	

TABLE 4

Water permeability of the gut tissues of three species of crabs as indicated by the percent THO saturation at 30 minutes and the hourly exchange fraction (K).

	Species	Number of Animals	Salinity Acclim. (‰S.W.)	Salinity Acute Treat. (‰S.W.)	Mean % Sat. (1 hr.)		Std. Error	K
1.	C. irroratus	6	100	100	5.45	±	0.740	1.1107
2.	C. irroratus	6	100	10	4.91	±	0.784	
3.	C. irroratus	6	40	40	2.28	±	0.314	0.0454
4.	C. irroratus	6	40	4	2.18	±	0.281	
5.	C. sapidus	6	100	100	5.00	±	0.383	0.1012
6.	C. sapidus	6	100	10	4.01	±	0.2981	
7.	C. sapidus	6	40	40	2.76	±	0.373	0.0551
8.	C. sapidus	6	40	4	2.45	±	0.318	
9.	L. emarginata	6	100	100	2.93	±	0.259	0.0590
10.	L. emarginata	6	100	10	3.03	±	0.229	

TABLE 5A

Effects of acclimation to 40% sea water of the gill, gut, and muscle tissues of *C. sapidus* and *C. irroratus*.

	Experimental Conditions			Tissue	Δ% Sat.	P
1.	*C. irroratus* 100% acclim.	vs	*C. irroratus* 40% acclim.	Gill	48.7	<.001
2.	*C. sapidus* 100% acclim.	vs	*C. sapidus* 40% acclim. (summer)	Gill	39.4	<.001
3.	*C. sapidus* 100% acclim.	vs	*C. sapidus* 40% acclim. (winter)	Gill	9.4	<.05
4.	*C. irroratus* 100% acclim.	vs	*C. irroratus* 40% acclim.	Gut	3.2	<.001
5.	*C. sapidus* 100% acclim.	vs	*C. sapidus* 40% acclim. (summer)	Gut	2.2	<.001
6.	*C. sapidus* 100% acclim.	vs	*C. sapidus* 40% acclim. (winter)	Gut	0.6	<.05
7.	*C. irroratus* 100% acclim.	vs	*C. irroratus* 40% acclim.	Muscle	23.3	>.05
8.	*C. sapidus* 100% acclim.	vs	*C. sapidus* 40% acclim.	Muscle	27.6	<.05

one half of the values at 100%. The calculated K values (hourly water exchange rates) are also different for the different salinities of acclimation (Table 3,4). Gills of C. irroratus exhibit K values of 1.55 at 100% acclimation and 0.35 at 40% acclimation, while gills of C. sapidus have water exchange rates of 1.33 and 0.33 at 100% and 40% salinity of acclimation respectively. Thus, C. irroratus and C. sapidus can reduce their water exchange or the permeability of their tissues upon acclimation to a lower salinity.

In the gill and gut tissues of the three species of crabs tested, the influx of THO is the same for the tissues subjected to the control salinity (100% or 40% sea water) as for the tissues treated with water at the stress salinity (10% or 4% sea water) ($P > .05$) (Tables 2&3, ln 1&2, 3&4, 5&6, 7&8, 9&10; Table 5B, ln 1-5, 6-10; Fig. 2,3). Thus there are no changes in the permeability of gill and gut to water during an osmotic stress.

Multi-factor analysis of variance showed that, in the case of the gill tissue, there are no interspecific differences in water permeability ($P > .05$) among the three species of decapods studied at 100% salinity of acclimation (Table 3, ln 1, 5, 9; Table 5C, ln 1-3; Fig. 2). During summer under these salinity conditions the THO influxes are the same for C. irroratus, C. sapidus and L. emarginata. At the 40% salinity of acclimation, gills of C. irroratus and C. sapidus also exhibit the same water permeability ($P > .05$) (Table 3, ln 3, 7; Table 5C, ln 4; Fig.2). In most instances, there are also no interspecific differences in permeability to THO of the gut tissues. Thus, the influx of THO across the gut of C. sapidus is the same as

the influx across the gut of C. irroratus ($P > .05$) when the crabs are acclimated either to 100% or 40% sea water (Table 4, ln 1&5, 3&7; Table 5C, ln 5, 8). However, during this set of experiments, the gut of L. emarginata was less permeable than the guts of the other two species acclimated to 100% sea water ($P < .001$) (Table 4, ln 1, 5, 9; Table 5C ln 6, 7; Fig. 3).

Results of the time course experiments show that the uptake of THO across the gills is not statistically higher ($P > .05$) after 2 hours than after 1 hour. Uptake across the gut is also similar after 30 minutes, 1 hour and 2 hours ($P > .05$). In almost all cases the % saturation values increased with time; however, those increases were small and the variability within each acclimation group was high enough to make the differences not statistically significant.

During the course of experiments on the effects of extracts from neuroendocrine tissues, control measurements were made of THO influx across the gill and gut tissues without extracts being added. Comparison of these data with data collected during the experiments on osmotic stress reveal some interesting results (Tables 5D, 6). The mean % saturation values of the gills of C. sapidus acclimated to 100% sea water in the winter is 16.08; while during the summer, the mean % saturation value for the gills under the same experimental conditions is 67.83 (Table 6, ln 1, 2). This relationship is the same for tissues of the two species of crabs that were tested in both seasons. The % saturation values of the gills and the guts of both C. sapidus and L. emarginata are lower in the experiments conducted in the winter (Tables 5D, 6). However the relationship between the THO influx and

TABLE 5B

Effects of anosmotic stress on the gill, gut and muscle tissues of the three species of crabs.

	Experimental Conditions			Tissue	4% Sat.	P
1.	C. irroratus 100% control	vs	C. irroratus 10% stress	Gill	12.2	>.05
2.	C. sapidus 100% control	vs	C. sapidus 10% stress	Gill	4.6	>.05
3.	L. emarginata 100% control	vs	L. emarginata 10% stress	Gill	15.3	>.05
4.	C. irroratus 40% control	vs	C. irroratus 4% stress	Gill	3.3	>.05
5.	C. sapidus 40% control	vs	C. sapidus 4% stress	Gill	0.3	>.05
6.	C. irroratus 100% control	vs	C. irroratus 10% stress	Gut	0.5	>.05
7.	C. sapidus 100% control	vs	C. sapidus 10% stress	Gut	0.9	>.05
8.	L. emarginata 100% control	vs	L. emarginata 10% stress	Gut	0.1	>.05
9.	C. irroratus 40% control	vs	C. irroratus 4% stress	Gut	0.1	>.05
10.	C. sapidus 40% control	vs	C. sapidus 4% stress	Gut	0.3	>.05
11.	C. irroratus 100% control	vs	C. irroratus 10% stress	Muscle	52.2	<.05
12.	C. sapidus 100% control	vs	C. sapidus 10% stress	Muscle	17.0	<.05
13.	L. emarginata 100% control	vs	L. emarginata 10% stress	Muscle	40.0	<.05
14.	C. irroratus 40% control	vs	C. irroratus 4% stress	Muscle	69.4	<.001
15.	C. sapidus 40% control	vs	C. sapidus 4% stress	Muscle	52.1	<.001

TABLE 5C

Interspecific comparisons of the water permeability of the gill and gut tissues of the three species of crabs.

	Experimental Conditions			Tissue	Δ% Sat.	P
1.	C. irroratus 100%	vs	C. sapidus 100%	Gill	10.9	>.05
2.	C. irroratus 100%	vs	L. emarginata 100%	Gill	3.4	>.05
3.	C. sapidus 100%	vs	L. emarginata 100%	Gill	7.6	>.05
4.	C. irroratus 40%	vs	C. sapidus 40%	Gill	1.7	>.05
5.	C. irroratus 100%	vs	C. sapidus 100%	Gut	0.5	>.05
6.	C. irroratus 100%	vs	L. emarginata 100%	Gut	2.5	<.05
7.	C. sapidus 100%	vs	L. emarginata 100%	Gut	2.1	<.05
8.	C. irroratus 40%	vs	C. sapidus 40%	Gut	0.5	>.05

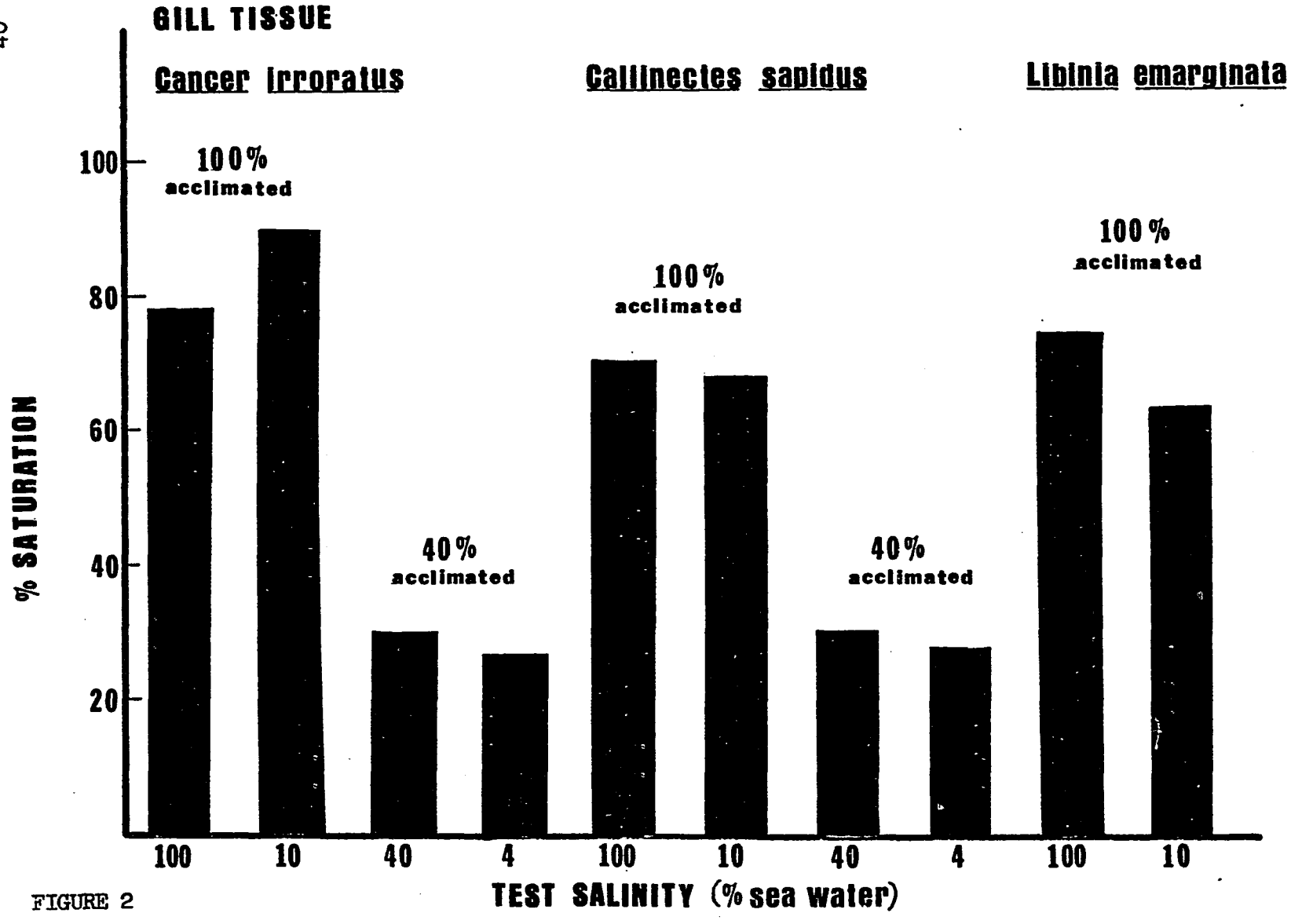

GILL TISSUE
Cancer irroratus
Callinectes sapidus
Libinia emarginata
100
80
60
40
20
% SATURATION
100% acclimated
40% acclimated
100% acclimated
40% acclimated
100% acclimated
100
10
40
4
100
10
40
4
100
10
TEST SALINITY (% sea water)

FIGURE 2

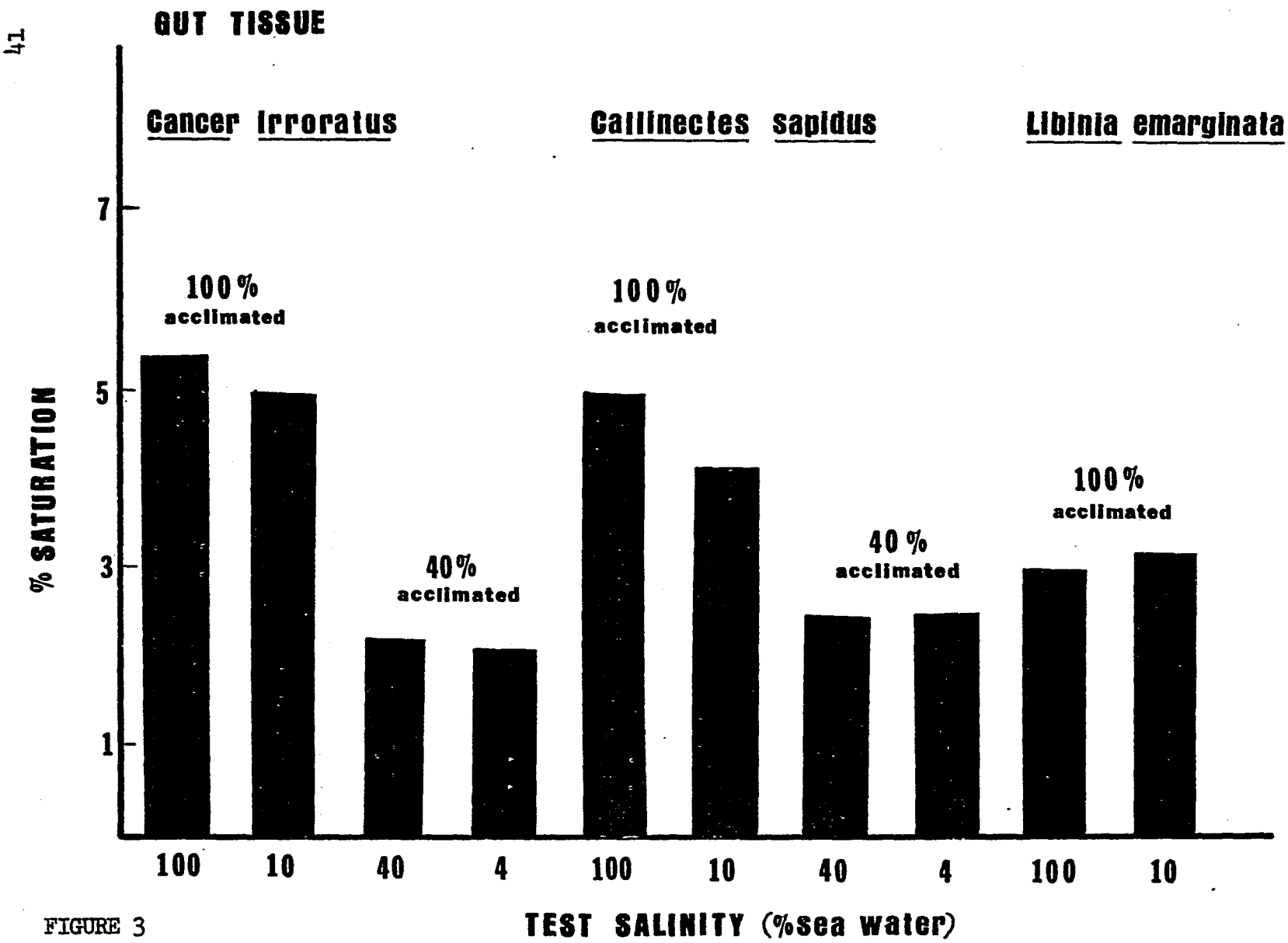

GUT TISSUE
Cancer irroratus
Callinectes sapidus
Libinia emarginata
% SATURATION
7
5
3
1
100 % acclimated
40 % acclimated
100 % acclimated
40 % acclimated
100 % acclimated
100
10
40
4
100
10
40
4
100
10
TEST SALINITY (%sea water)

FIGURE 3

the salinity of acclimation remains the same. During both seasons the water permeability of the gill and gut tissues of crabs acclimated to 40% sea water is significantly lower ($P < .05$) than the tissues of crabs acclimated to 100% sea water (Table 5A, ln 2, 3, 5, 6; Table 6).

A comparison of the interspecific differences in water permeability also yields some interesting results. During the summer experiments, there were no significant differences ($P > .05$) between the water permeability of the gills of C. sapidus and L. emarginata; while the movement of THO across the gut was lower in L. emarginata ($P < .001$) (Table 5C, ln 3, 7; Table 6). In the winter experiments, the gill and gut tissues of L. emarginata exhibited significantly higher % saturation values ($P < .05$) than the tissues of C. sapidus.

The results of the % saturation values for the muscle tissues are presented in Table 7 and Figs. 4, 5 and 6. For all three species, the muscle tissue that was osmotically stressed exhibited % saturation values that are significantly higher ($P < .05$) than the non-stressed tissue (Table 5B, ln 11-15; Table 7, ln 1&2, 3&4, 9&10). Thus, an osmotic stress of 10% of the original salinity increased the influx of THO into the tissue.

The % saturation values for the muscle tissues from C. irroratus acclimated to 100% sea water were not significantly different from the muscle tissue of those acclimated to 40% sea water ($P > .05$) (Table 5A, ln 7; Table 7, ln 1, 3; Fig. 4). However, in C. sapidus, muscle from crabs acclimated to 100% sea water exhibited THO influxes that were significantly lower than the THO influxes in muscles from crabs acclimated to 40% sea water ($P < .05$) (Table 5A, ln 8; Table 7, ln 5, 7; Fig. 5).

TABLE 5D

Effects of season on the water permeability of the gill and gut tissues of *C. sapidus* and *L. emarginata*.

	Experimental Conditions			Tissue	Δ% Sat.	P
1.	*C. sapidus* 100% summer	vs	*C. sapidus* 100% winter	Gill	51.7	<.001
2.	*C. sapidus* 40% summer	vs	*C. sapidus* 40% winter	Gill	21.7	<.001
3.	*L. emarginata* 100% summer	vs	*L. emarginata* 100% winter	Gill	46.6	<.001
4.	*C. sapidus* 100% summer	vs	*C. sapidus* 100% winter	Gut	3.5	<.001
5.	*C. sapidus* 40% summer	vs	*C. sapidus* 40% winter	Gut	1.8	<.001
6.	*L. emarginata* 100% summer	vs	*L. emarginata* 100% winter	Gut	0.9	<.05

TABLE 6

Effects of season (in mean % saturation).

	Species	N	Salinity of Acclim. (%S.W.)	Tissue	Mean % Sat. (1 hr.)		Standard Error
1.	C. sapidus (Summer)	6	100	Gill	67.83	±	16.35
2.	C. sapidus (Winter)	25	100	Gill	16.08	±	2.78
3.	C. sapidus (Summer)	6	40	Gill	28.39	±	6.82
4.	C. sapidus (Winter)	16	40	Gill	6.66	±	0.98
5.	L. emarginata (Summer)	6	100	Gill	75.42	±	11.53
6.	L. emarginata (Winter)	25	100	Gill	28.80	±	4.3
7.	C. sapidus (Summer)	6	100	Gut	5.00	±	0.38
8.	C. sapidus (Winter)	25	100	Gut	1.49	±	0.15
9.	C. sapidus (Summer)	6	40	Gut	2.76	±	0.37
10.	C. sapidus (Winter)	16	40	Gut	0.93	±	0.15
11.	L. emarginata (Summer)	6	100	Gut	2.93	±	0.25
12.	L. emarginata (Winter)	25	100	Gut	2.03	±	0.19

TABLE 7

Water permeability of the muscle tissue of the three species of crabs.

	Species	N	Acclim. Sal. (‰S.W.)	Treatment Salinity (‰S.W.)	Mean % Sat. (Pergram)		Std. Error
1.	C. irroratus	5	100	100	133.6	±	11.5
2.	C. irroratus	5	100	10	185.8	±	8.6
3.	C. irroratus	5	40	40	110.3	±	22.4
4.	C. irroratus	5	40	4	179.7	±	24.8
5.	C. sapidus	6	100	100	124.4	±	8.5
6.	C. sapidus	6	100	10	141.3	±	10.6
7.	C. sapidus	6	40	40	152.0	±	11.6
8.	C. sapidus	6	40	4	204.1	±	9.1
9.	L. emarginata	5	100	100	159.4	±	13.5
10.	L. emarginata	5	100	10	199.3	±	11.5

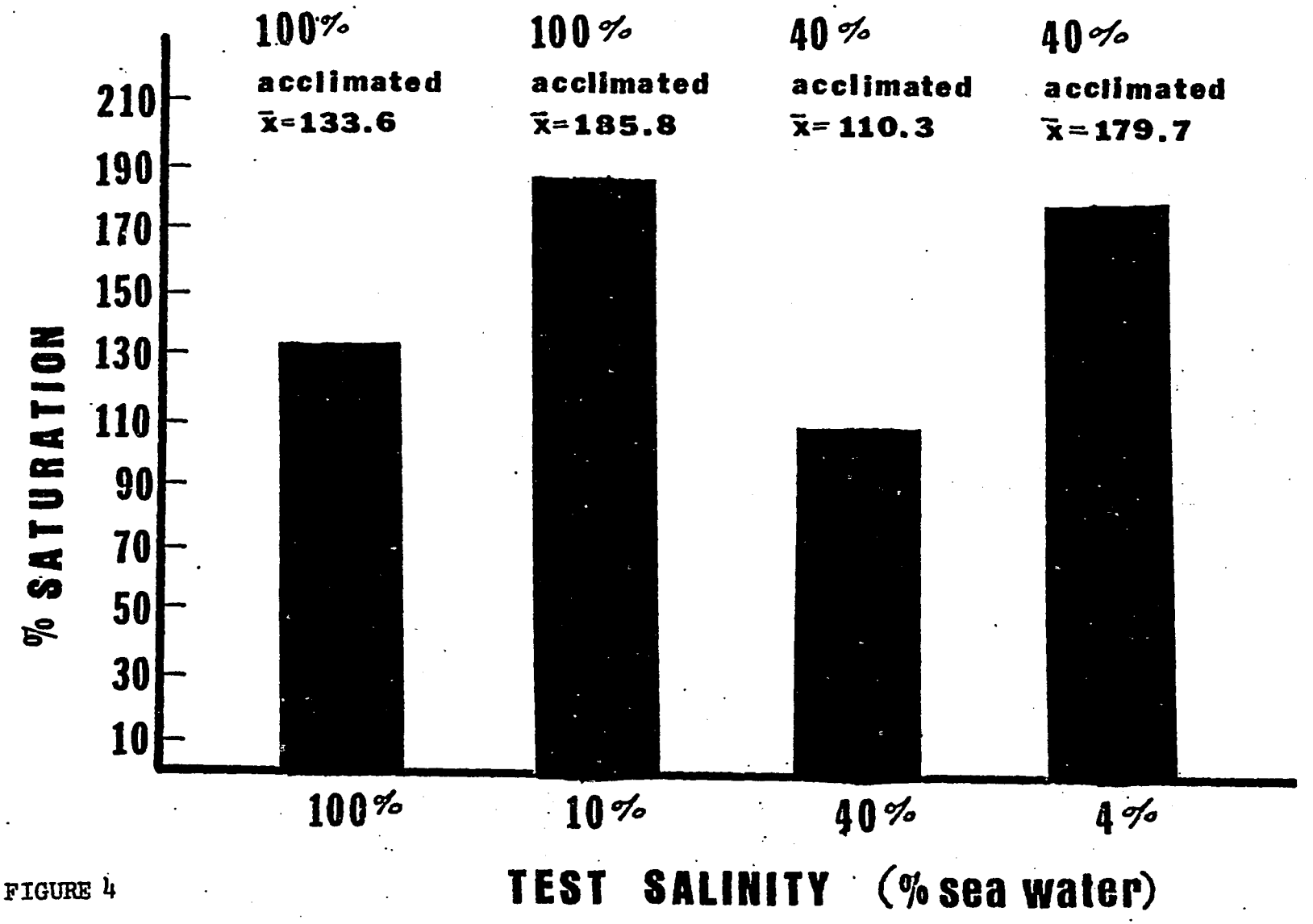

Cancer irroratus
100% acclimated x̄=133.6
100% acclimated x̄=185.8
40% acclimated x̄=110.3
40% acclimated x̄=179.7
% SATURATION
210
190
170
150
130
110
90
70
50
30
10
100%
10%
40%
4%
TEST SALINITY (% sea water)

FIGURE 4

MUSCLE TISSUE

Callinectes sapidus

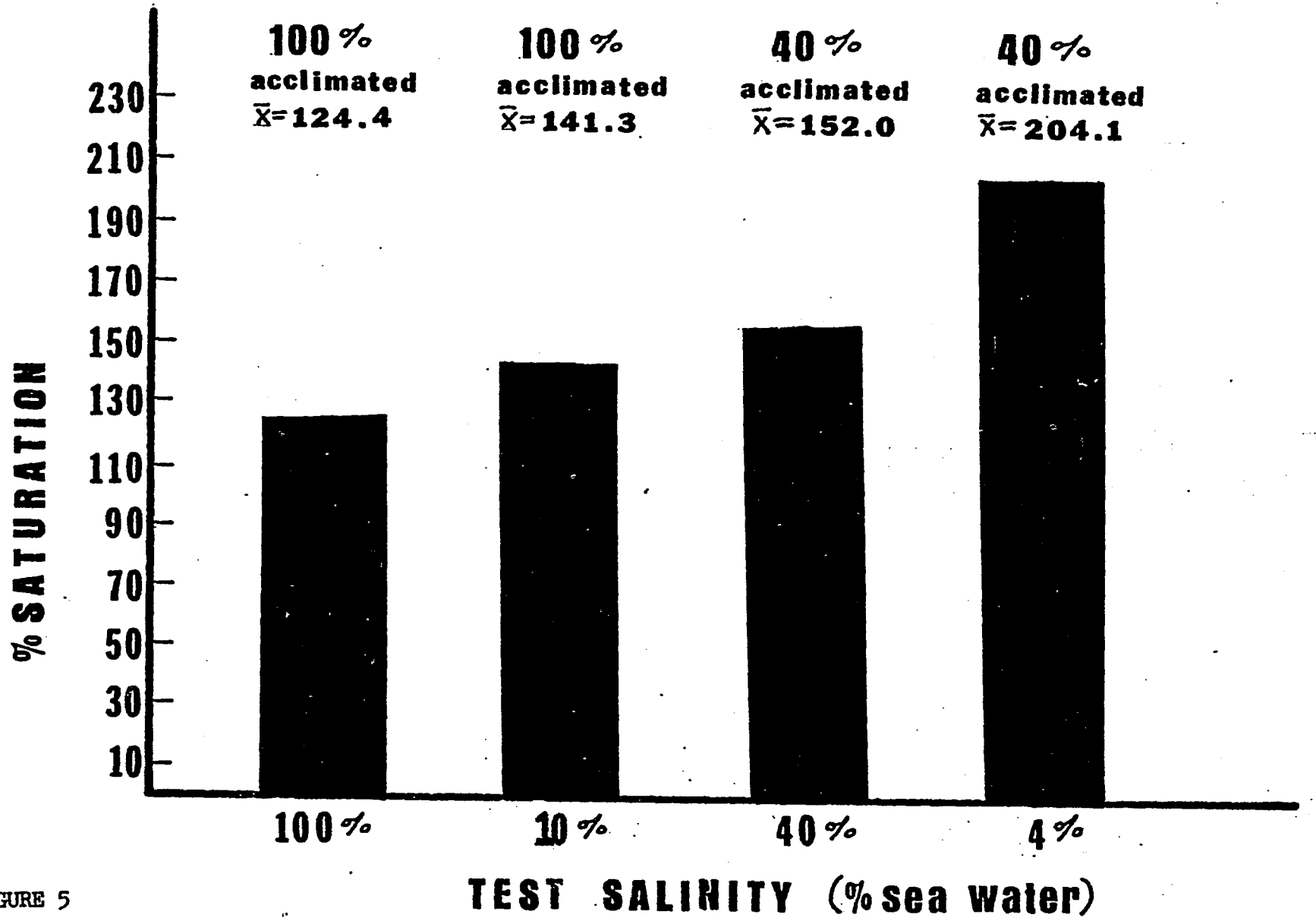


FIGURE 5

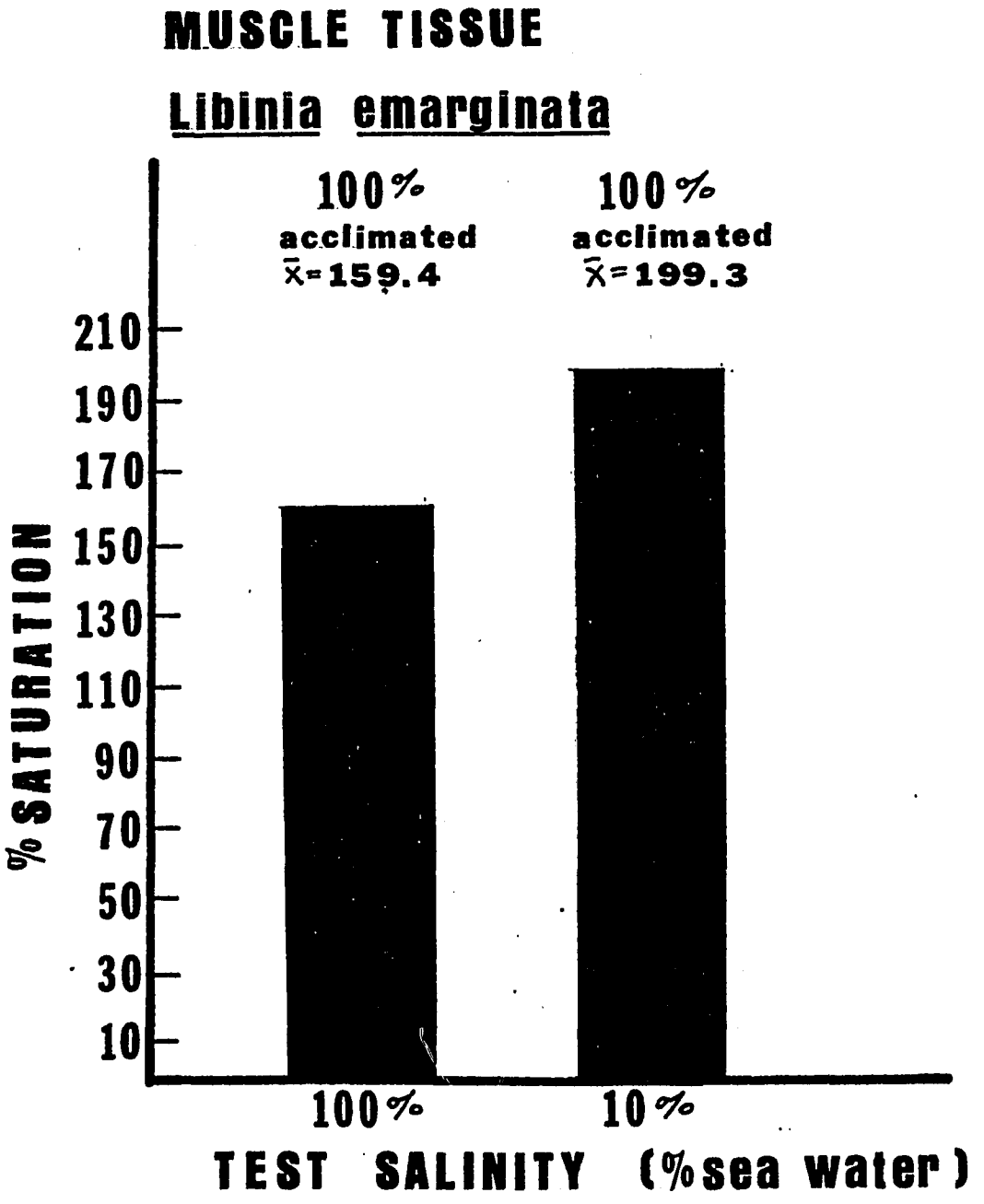

MUSCLE TISSUE
Libinia emarginata
100%
acclimated
x̄=159.4
100%
acclimated
x̄=199.3
% SATURATION
210
190
170
150
130
110
90
70
50
30
10
100%
10%
TEST SALINITY (%sea water)

FIGURE 6

Results of the time course studies on this set of experiments show that there are no significant increases ($P>.05$) in % saturation with the times tested (10, 20, 30, and 45 minutes). In almost all cases there was an increase in the amount of THO taken in; however, these increases were not statistically significant.

In general it may be stated that there is a reduction in water permeability of the gill and gut tissues when C. irroratus and C. sapidus are acclimated to a lower salinity. Seasonality also affects the water permeability of the gill and gut tissues in L. emarginata and C. sapidus, since the crabs tested in the winter exhibit lower permeability to water. However, subjecting tissues to an osmotic stress of 10% of the acclimation salinity does not alter the permeability of the gill and gut tissues. On the other hand, muscle tissue is affected by an osmotic stress, since a 10% stress causes an increase in THO uptake by the muscle tissue of the three crabs studied. There is, however, no generalized effect of the salinity of acclimation on the THO uptake by the muscle tissue.

EXPERIMENTS USING EXTRACTS OF NEUROENDOCRINE TISSUES

Gill and gut tissues of L. emarginata were treated with extracts made from tissues of L. emarginata acclimated to 100% sea water and C. sapidus acclimated to 40% sea water. The results of the THO influxes are presented in Table 8 and 9 and in Figures 7 and 8. Water permeability of the gills and guts treated with NM extracts was not significantly different ($P>.05$) from that

TABLE 8

Effects of neuroendocrine extracts on the water permeability of the gill tissue of the three species of crabs.

	Species	N	Acclim. Sal. (%S.W.)	Treatment	Mean % Sat. (1 hr.)	Std. Error
1.	C. sapidus	25	100	Water	16.08 ±	2.78
2.	C. sapidus	5	100	N.M. Extract from 2 C. sapidus 100%	14.27 ±	6.14
3.	C. sapidus	5	100	E.S. Extract from 4 C. sapidus 100%	11.86 ±	5.35
4.	C. sapidus	5	100	T.G. Extract from 4 C. sapidus 100%	3.95 ±	1.65
5.	C. sapidus	5	100	E.S. Extract from 2 C. sapidus 40%	9.73 ±	2.80
6.	C. sapidus	5	100	T.G. Extract from 2 C. sapidus 40%	6.96 ±	2.33
7.	C. sapidus	16	40	Water	6.66 ±	0.98
8.	C. sapidus	6	40	N.M. Extract from 2 C. sapidus 40%	7.07 ±	2.08
9.	C. sapidus	5	40	E.S. Extract from 4 C. sapidus 40%	5.37 ±	1.35
10.	C. sapidus	5	40	T.G. Extract from 4 C. sapidus 40%	4.63 ±	0.69
11.	L. emarginata	25	100	Water	28.8 ±	4.3
12.	L. emarginata	5	100	N.M. Extract from 2 L. emarginata 100%	28.2 ±	14.3
13.	L. emarginata	5	100	E.S. Extract from 4 L. emarginata 100%	27.7 ±	9.0
14.	L. emarginata	5	100	T.G. Extract from 4 L. emarginata 100%	16.3 ±	3.0
15.	L. emarginata	5	100	E.S. Extract from 2 C. sapidus 40%	13.8 ±	2.9
16.	L. emarginata	5	100	T.G. Extract from 2 C. sapidus 40%	6.9 ±	2.7

TABLE 9

Effects of neuroendocrine extracts on the water permeability of the gut tissue of the three species of crabs.

	Species	N	Acclim. Sal. (%S.W.)	Treatment	Mean % Sat. (1 hr.)	Std. Error
1.	C. sapidus	25	100	Water	1.49 ±	0.15
2.	C. sapidus	5	100	N.M. Extract from 2 C. sapidus 100%	1.54 ±	0.20
3.	C. sapidus	5	100	E.S. Extract from 4 C. sapidus 100%	1.83 ±	0.34
4.	C. sapidus	5	100	T.G. Extract from 4 C. sapidus 100%	0.96 ±	0.37
5.	C. sapidus	5	100	E.S. Extract from 2 C. sapidus 40%	1.13 ±	0.50
6.	C. sapidus	5	100	T.G. Extract from 2 C. sapidus 40%	1.41 ±	0.54
7.	C. sapidus	16	40	Water	0.93 ±	0.15
8.	C. sapidus	6	40	N.M. Extract from 2 C. sapidus 40%	1.31 ±	0.09
9.	C. sapidus	5	40	E.S. Extract from 4 C. sapidus 40%	0.66 ±	0.23
10.	C. sapidus	5	40	T.G. Extract from 4 C. sapidus 40%	0.53 ±	0.12
11.	L. emarginata	25	100	Water	2.03 ±	0.19
12.	L. emarginata	5	100	N.M. Extract from 2 L. emarginata 100%	2.57 ±	0.49
13.	L. emarginata	5	100	E.S. Extract from 4 L. emarginata 100%	1.74 ±	0.43
14.	L. emarginata	5	100	T.G. Extract from 4 L. emarginata 100%	0.95 ±	0.11
15.	L. emarginata	5	100	E.S. Extract from 2 C. sapidus 40%	1.37 ±	0.34
16.	L. emarginata	5	100	T.G. Extracts from 2 C. sapidus 40%	1.27 ±	0.34

GILL TISSUE

Libinia emarginata

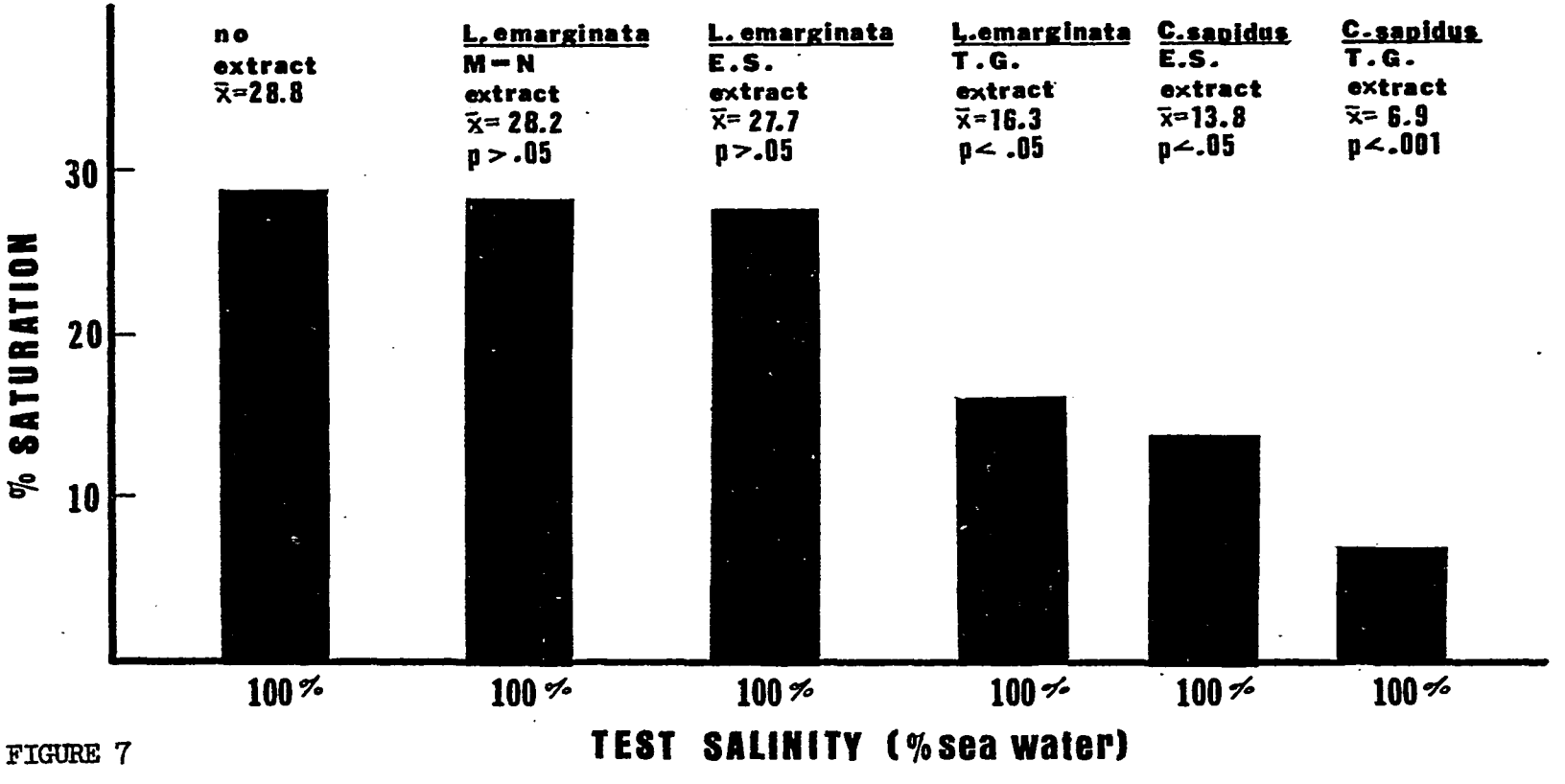


FIGURE 7

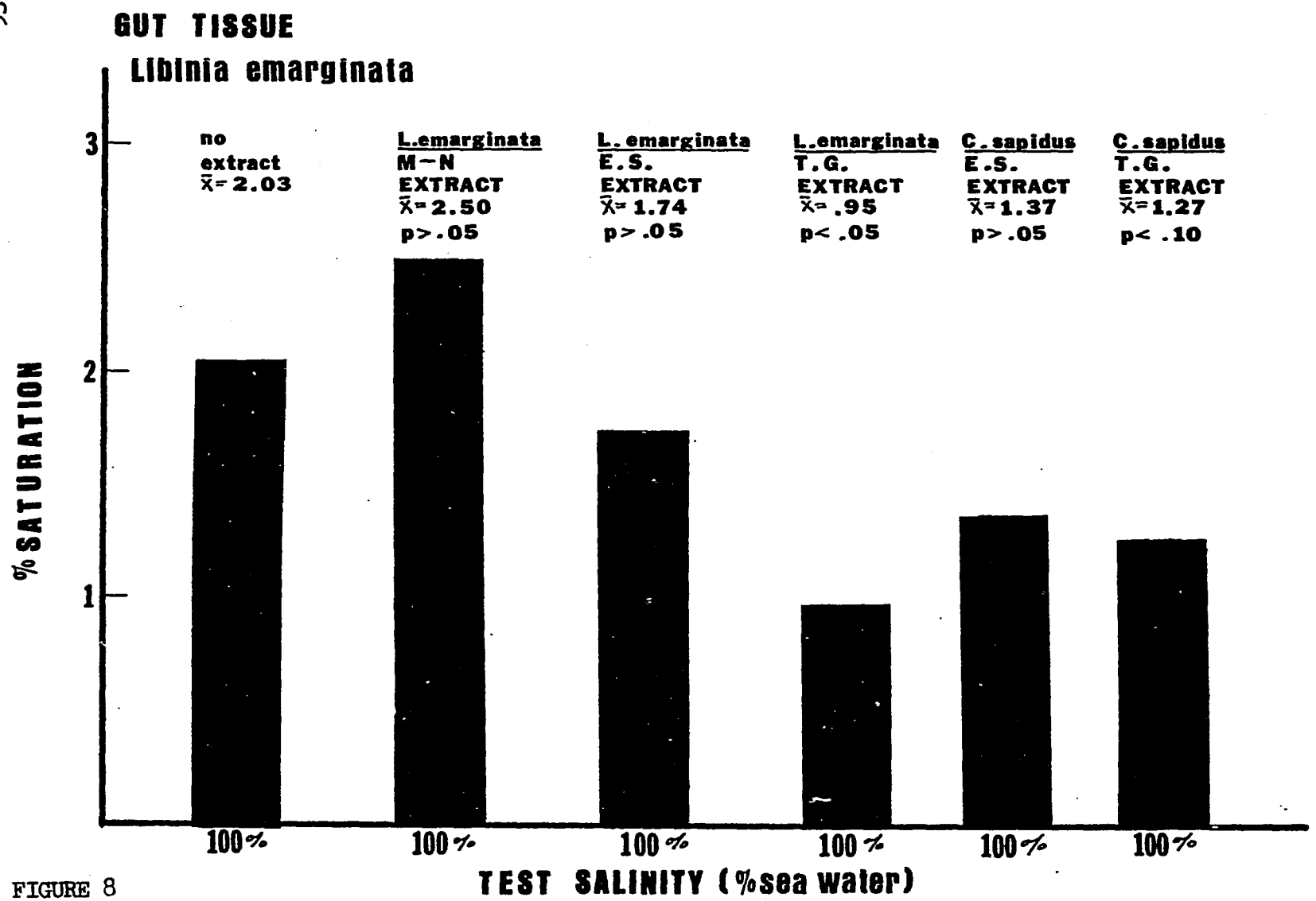

GUT TISSUE
Libinia emarginata
no extract x̄= 2.03
L.emarginata M—N EXTRACT x̄= 2.50 p>.05
L. emarginata E.S. EXTRACT x̄= 1.74 p>.05
L.emarginata T.G. EXTRACT x̄= .95 p< .05
C. sapidus E.S. EXTRACT x̄= 1.37 p>.05
C. sapidus T.G. EXTRACT x̄= 1.27 p< .10
% SATURATION
3
2
1
100%
100%
100%
100%
100%
100%
TEST SALINITY (%sea water)

FIGURE 8

of the gills and guts that did not receive extracts (Table 5E, ln 9, 22; Tables 8 and 9, ln 11, 12). Therefore, an alteration in permeability cannot be attributed to the presence of a factor in the non-neuroendocrine tissues of crabs. The gill and gut tissues did not exhibit a significant decrease ($P > .05$) in the THO influx when they were treated with extracts made from the ES of 4 L. emarginata (Table 5E, ln 11, 24; Tables 8 and 9, ln 13). However, the gill tissues do show a significant decrease ($P < .05$) in water permeability when they are treated with extracts made from the TGM of 4 L. emarginata or ES from 2 C. sapidus acclimated to 40% sea water (Table 5E, ln 10, 13; Table 8, ln 14, 15). The greatest reduction occurs when the gills are treated with the TGM extracts from 2 C. sapidus acclimated to 40% sea water (i.e., 28.8 to 6.9) ($P < .001$) (Table 5E, ln 12). Water permeability of the gut tissue was significantly reduced only when treated with extracts from the TGM of 4 L. emarginata ($P < .05$) (Table 5E, ln 23; Table 9, ln 14). Treatment of the guts with TGM or ES extracts from 2 C. sapidus acclimated to 40% sea water caused a reduction in permeability to water (i.e., 2.03 to 1.27 or 2.03 to 1.37). However, due to the variability of the tissue response, the water permeability of the guts treated with extracts from C. sapidus did not prove to be significantly lower ($P < .1$, $P > .05$) than the water permeability of the controls (Table 5E, ln 25, 26).

Gill and gut tissues of C. sapidus acclimated to 100% sea water were treated with extracts made from tissues of C. sapidus acclimated to 100% sea water and C. sapidus acclimated to 40% sea water. The %

saturation values are presented in Tables 8 and 9 and Figs. 9 and 10. The % saturation values of both gills and guts for the NM controls were not significantly different from the tissues that did not receive extracts ($P > .05$) (Table 5E, ln 1, 14; Tables 8 and 9, ln 1, 2). The water permeability of the gill tissues was reduced significantly when extracts of TGM from 4 C. sapidus acclimated to 100% sea water ($P < .001$) or TGM from 2 C. sapidus acclimated to 40% sea water ($P < .05$) were added (Table 5E, ln 2, 4; Table 8, ln 4, 6). Extracts of the ES of C. sapidus acclimated to both 100% ($P > .05$) and 40% sea water ($P < 0.1$) reduced the permeability; however, the differences were not significant (Table 5E, ln 3, 5; Table 8, ln 3, 5). The THO influx of the gut tissue was not reduced significantly ($P > .05$) by the addition of any of the extracts (Table 5E, ln 15-18; Table 9, ln 3-6). The TGM extract of C. sapidus acclimated to 100% sea water did reduce the permeability; however, the difference was not significant (Table 5E, ln 15).

The tissues of C. sapidus acclimated to 40% sea water were treated with extracts from the tissues of C. sapidus acclimated to 40% sea water (Tables 8 and 9; Figs. 11 and 12). The % saturation values of both the gills and guts do not vary significantly from the values of the water control ($P > .05$) with the addition of any of the extracts (TGM, ES, or NM) (Table 5E, ln 6-8, 19-21; Tables 8 and 9, ln 8-10). Extracts from the TGM and ES reduce the permeability to water but not significantly (Table 5E, ln 7, 8, 20, 21).

The water permeability of both the gill and gut tissues of C. sapidus and L. emarginata is affected by extracts made from the

TABLE 5E

Effects of neuroendocrine extracts on the water permeability of the gill and gut tissues of *C. sapidus* and *L. emarginata*.

	Experimental Conditions			Tissue	Δ% Sat.	P
1.	*C. sapidus* 100% control	vs	NM extract *C. sapidus* 100%	Gill	1.8	>.05
2.	*C. sapidus* 100% control	vs	TGM extract *C. sapidus* 100%	Gill	12.4	<.001
3.	*C. sapidus* 100% control	vs	ES extract *C. sapidus* 100%	Gill	4.2	>.05
4.	*C. sapidus* 100% control	vs	TGM extract *C. sapidus* 40%	Gill	9.1	<.05
5.	*C. sapidus* 100% control	vs	ES extract *C. sapidus* 40%	Gill	6.4	<.10
6.	*C. sapidus* 40% control	vs	NM extract *C. sapidus* 40%	Gill	0.4	>.05
7.	*C. sapidus* 40% control	vs	TGM extract *C. sapidus* 40%	Gill	2.0	>.05
8.	*C. sapidus* 40% control	vs	ES extract *C. sapidus* 40%	Gill	1.3	>.05
9.	*L. emarginata* 100% control	vs	NM extract *L. emarginata* 100%	Gill	0.6	>.05
10.	*L. emarginata* 100% control	vs	TGM extract *L. emarginata* 100%	Gill	12.5	<.05
11.	*L. emarginata* 100% control	vs	ES extract *L. emarginata* 100%	Gill	1.1	>.05
12.	*L. emarginata* 100% control	vs	TGM extract *C. sapidus* 40%	Gill	21.9	<.001
13.	*L. emarginata* 100% control	vs	ES extract *C. sapidus* 40%	Gill	15.0	<.05
14.	*C. sapidus* 100% control	vs	NM extract *C. sapidus* 100%	Gut	0.05	>.05
15.	*C. sapidus* 100% control	vs	TGM extract *C. sapidus* 100%	Gut	0.5	>.05
16.	*C. sapidus* 100% control	vs	ES extract *C. sapidus* 100%	Gut	0.3	>.05
17.	*C. sapidus* 100% control	vs	TGM extract *C. sapidus* 40%	Gut	0.08	>.05
18.	*C. sapidus* 100% control	vs	ES extract *C. sapidus* 40%	Gut	0.4	>.05
19.	*C. sapidus* 40% control	vs	NM extract *C. sapidus* 40%	Gut	0.5	>.05
20.	*C. sapidus* 40% control	vs	TGM extract *C. sapidus* 40%	Gut	0.4	>.05
21.	*C. sapidus* 40% control	vs	ES extract *C. sapidus* 40%	Gut	0.3	>.05
22.	*L. emarginata* 100% control	vs	NM extract *L. emarginata* 100%	Gut	0.5	>.05
23.	*L. emarginata* 100% control	vs	TGM extract *L. emarginata* 100%	Gut	1.1	<.05
24.	*L. emarginata* 100% control	vs	ES extract *L. emarginata* 100%	Gut	0.3	>.05
25.	*L. emarginata* 100% control	vs	TGM extract *C. sapidus* 40%	Gut	0.8	<.10
26.	*L. emarginata* 100% control	vs	ES extract *C. sapidus* 40%	Gut	0.7	>.05

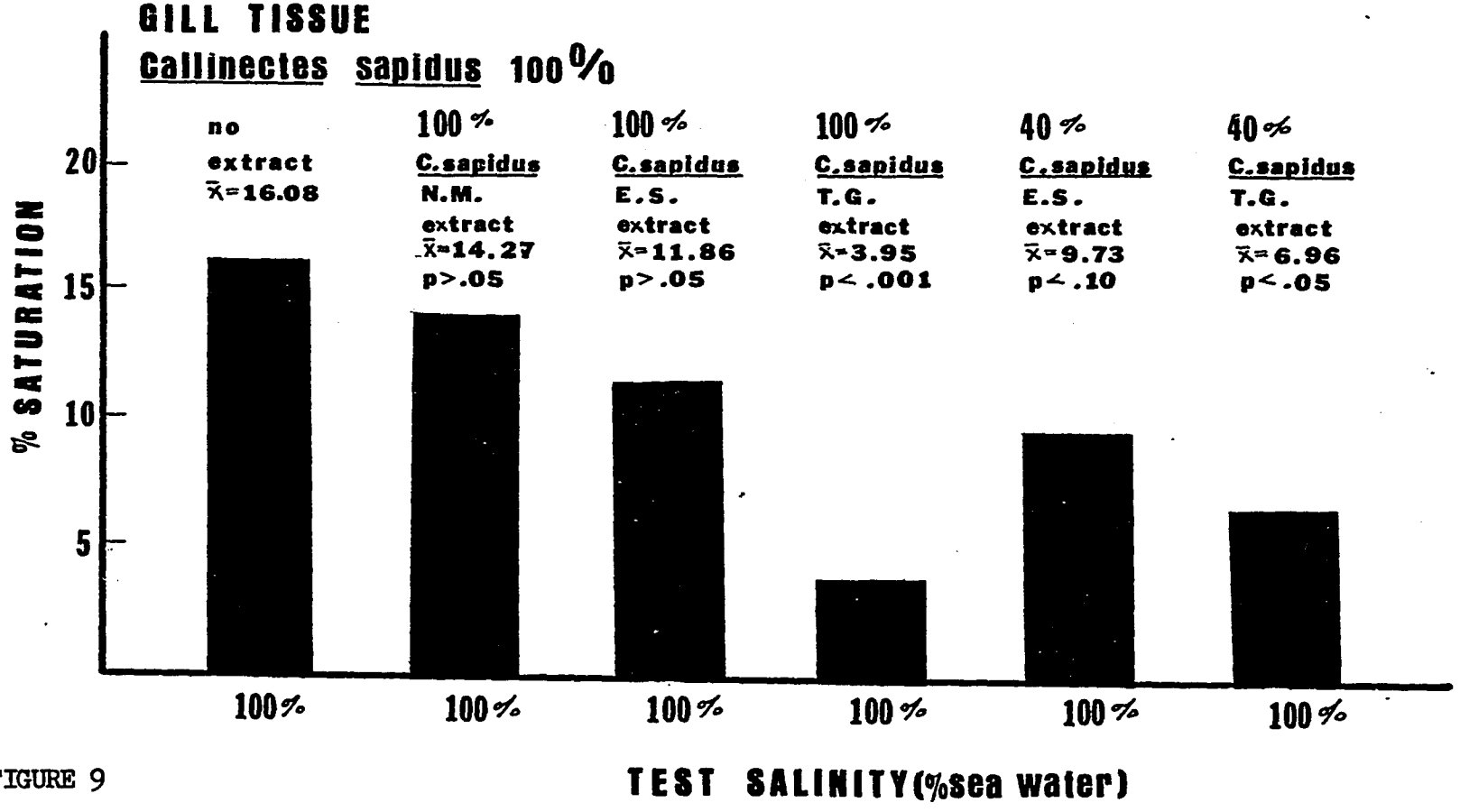

GILL TISSUE
Callinectes sapidus 100%
no extract x̄=16.08
100% C.sapidus N.M. extract x̄=14.27 p>.05
100% C.sapidus E.S. extract x̄=11.86 p>.05
100% C.sapidus T.G. extract x̄=3.95 p<.001
40% C.sapidus E.S. extract x̄=9.73 p<.10
40% C.sapidus T.G. extract x̄=6.96 p<.05
% SATURATION
20
15
10
5
100%
100%
100%
100%
100%
100%
TEST SALINITY(%sea water)

FIGURE 9

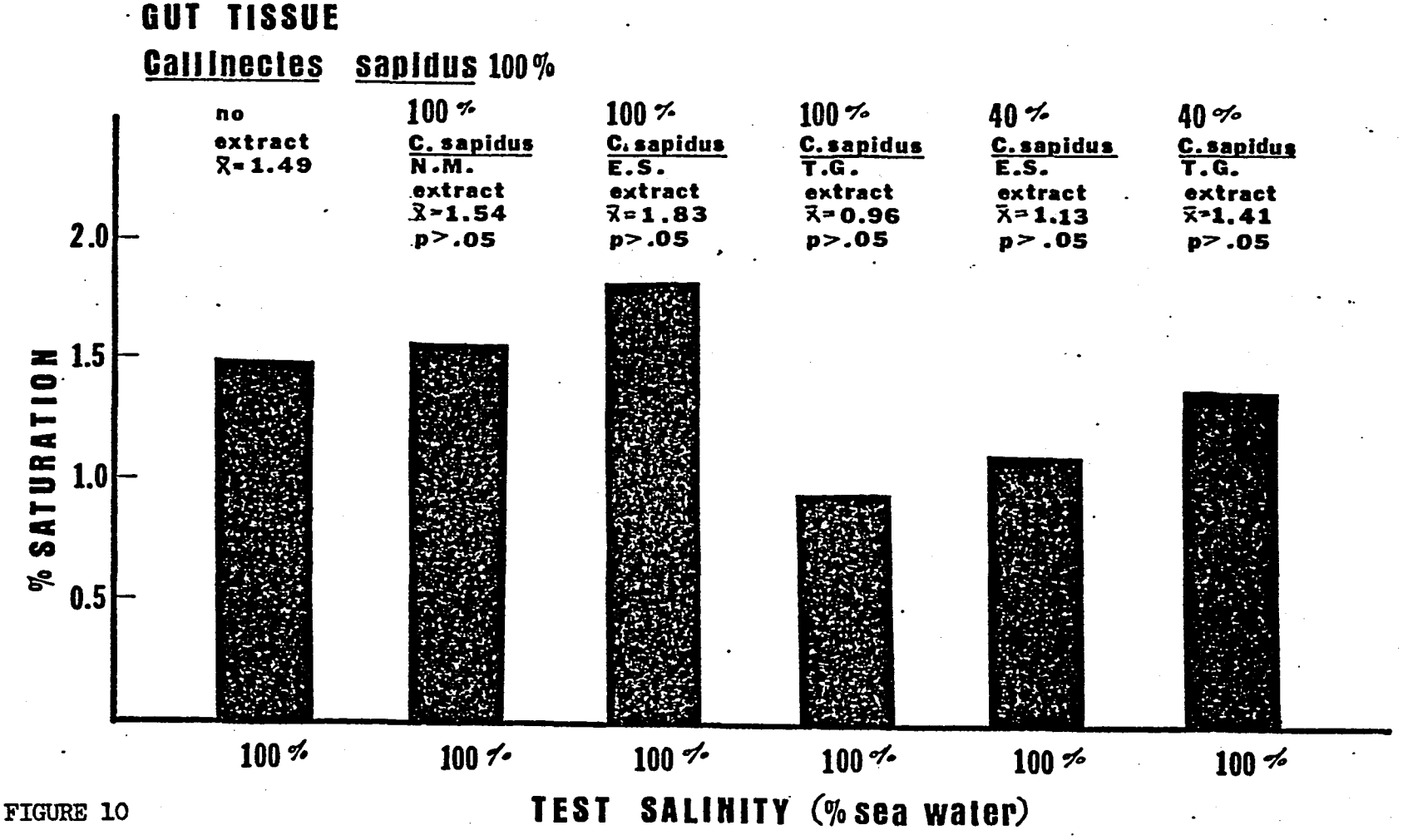

GUT TISSUE
Callinectes sapidus 100%
no extract x̄=1.49
100% C. sapidus N.M. extract x̄=1.54 p>.05
100% C. sapidus E.S. extract x̄=1.83 p>.05
100% C. sapidus T.G. extract x̄=0.96 p>.05
40% C. sapidus E.S. extract x̄=1.13 p>.05
40% C. sapidus T.G. extract x̄=1.41 p>.05
% SATURATION
2.0
1.5
1.0
0.5
100%
100%
100%
100%
100%
100%
TEST SALINITY (% sea water)

FIGURE 10

neuroendocrine tissues. TGM extracts appear to have the greatest effect on water permeability, lowering it as much as 21.9% of the original value. The greatest overall effect on water permeability occurs when the gills of an osmoconforming species, L. emarginata, are treated with extracts from an osmoregulating species, C. sapidus, that is acclimated to a lower salinity.

GILL TISSUE

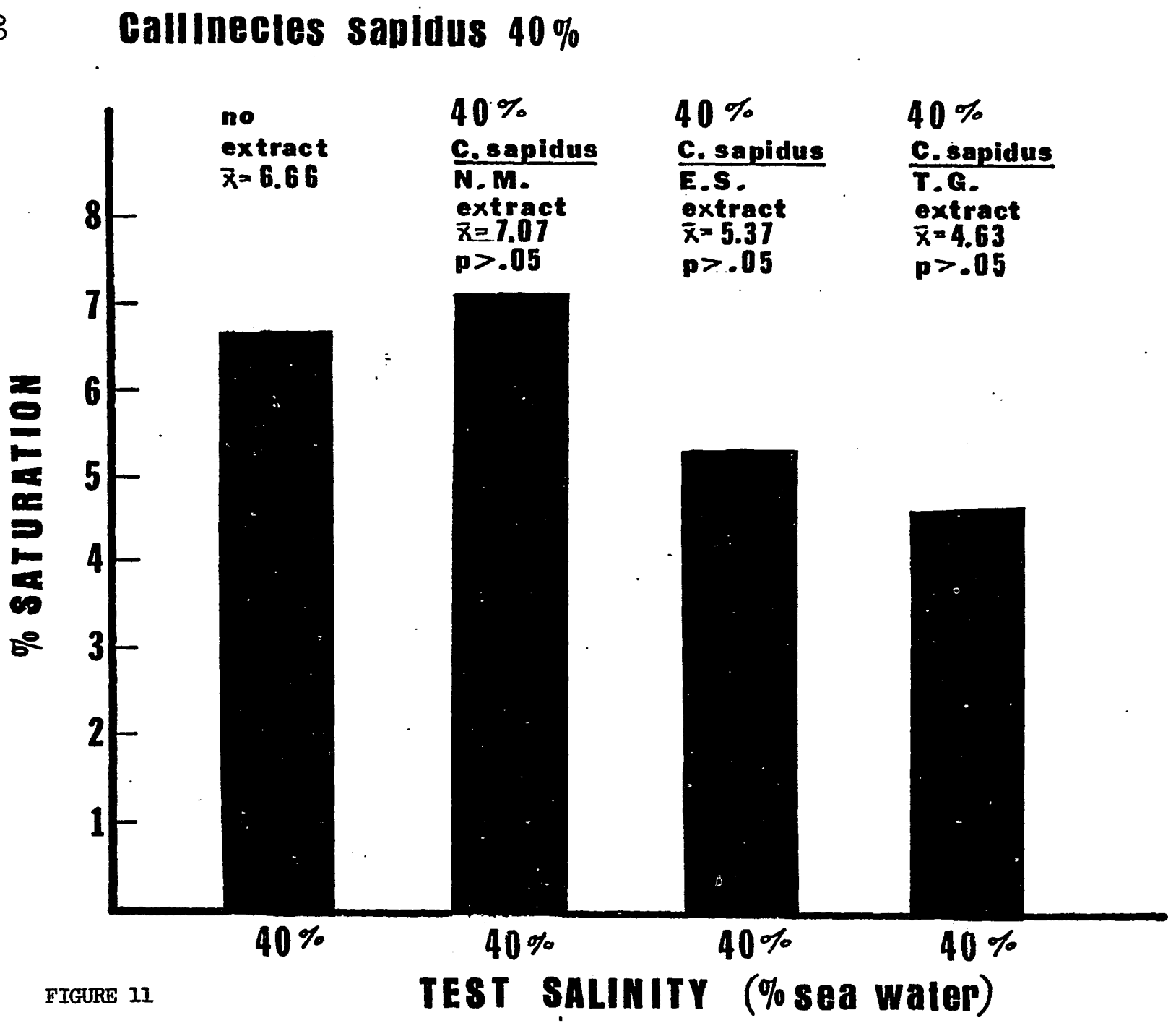


FIGURE 11

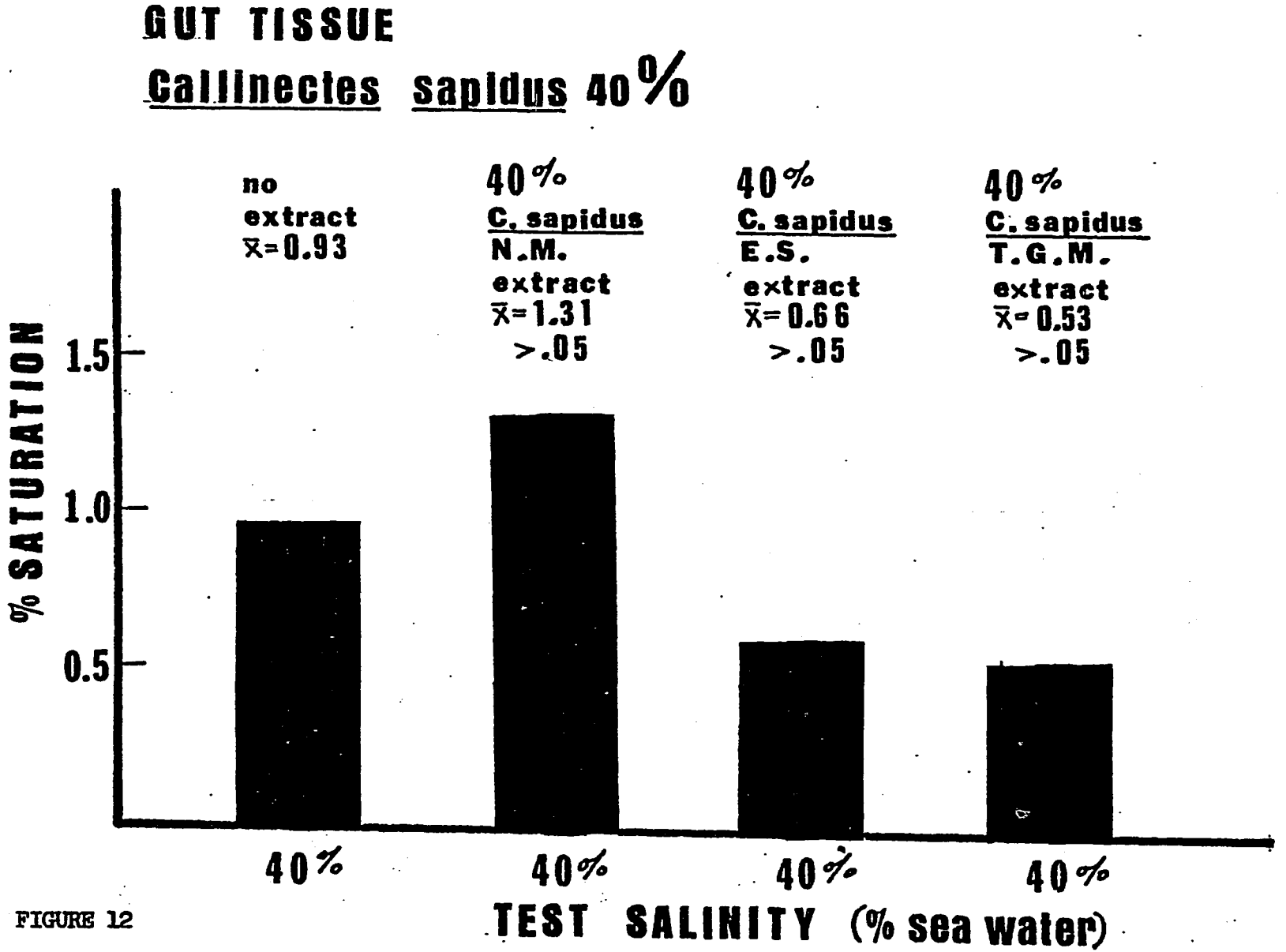

GUT TISSUE
Callinectes sapidus 40%
no extract
x̄=0.93
40% C. sapidus N.M. extract
x̄=1.31
>.05
40% C. sapidus E.S. extract
x̄=0.66
>.05
40% C. sapidus T.G.M. extract
x̄=0.53
>.05
% SATURATION
1.5
1.0
0.5
40%
40%
40%
40%
TEST SALINITY (% sea water)

FIGURE 12

DISCUSSION

SEASONAL EFFECTS

There are many studies in the literature that suggest seasonal variations in the physiological responses of decapod crustaceans. Mantel (1965) and Lynch et al. (1973) state that serum chloride, sodium, and osmotic concentrations of C. sapidus are higher in the winter and lower in the spring and summer months. Weber and Spaargaren (1970) found that temperature affected the osmoregulatory ability of Crangon crangon. Kamemoto (1976) states that seasonal cycles may contribute to the variations workers have observed in their studies of the neuroendocrinology of osmoregulation.

Comparison of the water permeability of the gill and gut tissues of C. sapidus and L. emarginata in the summer and winter also suggest a seasonal effect. The THO influxes were observed to be much lower across the tissues of winter animals than across the tissues of summer animals. The reduction of water permeability of C. sapidus coincides with the reduction of ATPase activity in the gill tissues that has been observed by Mantel and by Towle (personal communication). It also appears to correspond with a reduction in the general activity of this species during the winter months. Warner (1976), in his book on the natural history of C. sapidus, reports that when the water temperature drops in the winter, crabs are known to burrow into the mud and to cease almost all activity until late spring. The natural

history of L. emarginata is not as well known as that of C. sapidus. In general, however, L. emarginata inhabits deeper water and is a less active crab than C. sapidus. The seasonal reduction in permeability is not as great in L. emarginata as it is in C. sapidus and this may reflect the more sluggish life style and deeper habitat of L. emarginata.

Mantel (1968) has demonstrated that the neuroendocrine levels at different stages of the molt cycle affect the water permeability of the isolated gut tissue of G. lateralis. Hinsch (1972) states that female L. emarginata molt in the laboratory from late August through September. C. sapidus is also not known to molt in the winter in northern latitudes. Therefore, it may be that hormones involved in maintaining animals in the C-4 or intermolt stage during the winter may affect the water permeability of ionically active tissues.

The osmolality values for C. sapidus do not indicate the seasonal shifts suggested by Lynch (1973) and Mantel (1965). There are no significant differences between the seasonal osmolalities in this study. However, Lynch made measurements on freshly caught, naturally occuring populations of C. sapidus. Mantel maintained the animals in the laboratory for 4-5 days before experimentation. The crabs in the present study were maintained in the test salinities at 15° C for a minimum of two weeks before the hemolymph was sampled. This may account for the lack of seasonal differences between the hemolymph and the medium. Also the animals that Lynch sampled came from populations in water that had a mean osmolality which was lower that that of water of the salinity used in the present study. Therefore,

the osmolality of the hemolymph of crabs from natural populations in more saline water is not well documented.

THO DIFFUSION WITH AND WITHOUT AN OSMOTIC GRADIENT

ACCLIMATION

The results of this study show that in both seasons osmotically active tissues such as the gill and gut exhibit a reduction in permeability when either C. irroratus or C. sapidus is acclimated to a lower salinity. It is difficult to make comparisons of these results with those found in the literature because most of the other data were obtained from whole animals. However, the reduction in tissue permeability to water upon acclimation agrees with the reduction in whole animal permeability to water observed by workers on other euryhaline decapods. Smith (1967) and Capen (1972) have both reported that R. harrisii significantly lowers its apparent permeability to water on acclimation to lower salinities. Smith (1970) found that C. maenas shows a reduction in apparent permeability in 50% sea water and Smith and Rudy (1972) state that H. nudus also shows a reduction when acclimated to 60% sea water.

Capen performed a study that examined the D_2O uptake by the body and the isolated gills of the decapod R. harrisii. He found that when crabs are acclimated to 10% sea water the permeability to water of the whole animal is reduced compared to the permeability of the animal in 75% sea water. However, the isolated gill preparations show the

opposite results; that is, gills in the lower salinity show increased water uptake. The contradiction between Capen's data on the isolated gills and the present data may be explained by the methods that were used. Capen's method consisted of suspending a whole gill in a D_2O labeled bath for a period of time and measuring the amount of label that accumulated in the whole gill tissue. This is essentially the same method that was used for the muscle tissue in the present study. Therefore, Capen was measuring the diffusional water uptake into the intracellular and extracellular spaces of the whole gill. A greater osmotic gradient exists between the external medium and the gill tissue in an osmoregulating crab acclimated to 10% sea water than in a crab that is osmoconforming to 75% sea water. Therefore, high water uptake by the cells of the gills in the lower salinity might be expected because of the greater disparity in osmolality between the cells and the external medium. The gill tissue in the present study is perfused in such a manner that the diffusional influx of THO across the gill membrane and not the water uptake by the cells is being measured. This is more reflective of the system as it operates in vivo, as it enables changes in the water movements across the gill to be quantified.

The results of the percent saturation values for the muscle tissue at the different acclimation salinities do not suggest any clear patterns. There is no significant difference in the THO uptake in C. irroratus acclimated to 100% or 40% sea water. The cells of the muscle tissue of this species may be able to operate physiologically only if there is a relatively constant amount of water present in the

tissue. The percent saturation values of the muscle tissue of C. sapidus acclimated to 40% sea water are significantly higher than the values of C. sapidus acclimated to 100% sea water. Perhaps the cells of this euryhaline decapod are able to tolerate a greater amount of water either in or around them and still operate efficently. There is also the possibility that the in vitro muscle tissue is not exposed to neuroendocrine factors that may control the permeability in vivo in an osmoregulating crab acclimated to 40% sea water.

STRESS

The muscle tissue of the three species of decapods responded to an osmotic stress by significantly increasing the amount of THO that was taken in (Table 5B). This response is predictable based on the previous work that has been done on isolated muscle fibers and axons of C. sapidus. Lang and Gainer (1969) in their classic paper, observed that isolated muscle fibers from C. sapidus placed in hyposmotic media swell within the first five minutes to a peak and gradually, over several hours, return toward their normal volume. Gerard (1975) and Gerard and Gilles (1972) have demonstrated the same pattern of volume regulation on isolated axons of the same species. The subsequent decrease in cell volume appears to be due to the loss of free amino acids (Potts and Parry, 1964; Schoffeniels and Gilles, 1970; Gerard and Gilles, 1972). The changes in amino acid concentrations are the result of an active process that is responsible for the regulation of cellular volume during an osmotic stress. Schoffeniels and Gilles

(1970) do not believe that cellular volume regulation is under hormonal control; however, Tucker and Costlow (1975) suggest that ES factors may affect the free amino acid changes in the larvae of C. sapidus. The time course of the present experiment (10-45 minutes) was shorter than the several hours needed to observe the return toward normal volume. Thus, all of the percent saturation values of the muscles in the stressed osmolality are significantly above the values of the muscles in the control osmolality.

The present set of experiments measured total uptake of THO by the whole piece of muscle tissue. It was impossible to distinguish whether the THO was taken up intracelluarly or extracellularly. However, work in the literature shows that the extracellular spaces do not vary upon exposure to hyposmotic media. Seibers and Lucu (1973) demonstrated that the extracellular spaces of C. maenas do not differ significantly in response to external salinities. Lockwood and Inman (1973 A, B) also found that the extracellular space of the amphipod G. duebeni remained constant after the animals had been acclimated to a wide range of salinities. Lang and Gainer (1969) examined the extracellular space of the isolated muscle fibers that were maintained under different salinity conditions for 6 hours at 20°C and found the spaces to be unchanged. These data suggest that the increase in THO influx into the muscle tissue of the three species of decapods was due to the initial increase in cell volume because of the hyposmotic stress.

In all three species of crabs that were examined, there are no significant changes in water movement across the gill or gut tissues when they are exposed to a salinity stress. The difference between

the response of the muscle tissue and that of the gill and gut tissues may be explained by the methods that were used. The method that was employed on the muscle tissue measured the THO uptake of the cells of the muscle. The method that was used on the gill and gut tissues measured the movement of THO across the epithelium from the medium to the hemolymph side of the gills and from the lumen to the hemolymph side of the gut. The method did not measure the THO uptake by the cells of these tissues. The cells of the gill and gut have a cuticle that separates the cell membrane from the external environment. However, the muscle tissue has no cuticle that might affect the diffusional permeability of the tissues. The cellular membranes of tissues that have a cuticle may behave differently or with a different time course from tissues that do not possess a cuticle.

Capen (1972) showed that R. harrisii acclimated to 75% sea water and stressed with 10% sea water exhibited a decrease in water uptake by the whole animal. However, the present experiments show that epithelial tissues such as the gill and gut do not respond to an osmotic stress by changing their diffusional permeability to water. The individual cells of the epithelial tissues may be responding as the cells of the muscle tissue do by increasing their cell volume and then lowering it by reducing the amino acid concentrations (Schoffeniels and Gilles 1970; Gerard and Gilles, 1972). However, this process is undetectable in the present experimental design. Also, the free amino acid changes in the gill tissue have been reported to be small (Gerard and Gilles, 1972) and Haberfield and Hass (1975) have shown that they may not occur within two hours. The lack of osmotic

response to an osmotic stress in the isolated tissue preparation may occur because there is no exposure of the tissues to the central nervous system or to the hemolymph, where factors that may affect the permeability of the membranes might be found. Therefore, it is possible that changes in the permeability to water across the gill and gut after an osmotic stress may occur in vivo but not in vitro.

SITE OF WATER UPTAKE

Hannen and Evans (1973) and Capen (1972) have suggested that the gills are responsible for the differences in water uptake in different salinities. This suggestion seems to be supported by the present experiments since values for hourly water exchange (K) of several whole animals (e.g., C. meanas, R. harrisii, H. nudus) compare favorably to the values for the isolated gills of C. irroratus and C. sapidus. However, Cornell (1974) has reported a K value of 8.49 for whole L. emarginata in 100% sea water. In the present study, the isolated gills have a K value of 1.39 at the same salinity. These findings do not support the hypothesis that the gills are the main site for water uptake in this crab. However, the observed increase in permeability of the carapace with increased stenohalinity (Gross, 1957) may account for some of the differences between the whole body and the gill values. L. emarginata, an osmoconforming, stenohaline crab would be expected to have a more permeable carapace. The gills, therefore, would account for less of the total percentage of the water taken in. As osmoregulators, C. irroratus and C. sapidus would have less

permeable exoskeletons; thus their gills would have exchange rates that would more closely approximate the rates for the whole body.

TIME COURSE

In all crabs examined, there are no significant changes in THO movement with time for the gill, gut and muscle tissues. In most cases, the THO influxes increased with time. However, the increases were small, and there was high within-group variation. Some of the variability of the percent saturation values of the muscle tissue results from the treatment of the tissue. Muscles were dissected away from the carapace and cut into several pieces. This manipulation causes damage to the cells and allows cut fibers to be exposed to the experimental media. The small increases of percent saturation values with time suggest that the perfusion system of the epithelial tissues and the cells of the muscle tissue were tending toward a steady state based on the characteristics of the membranes involved. It appears that the maximum amount of uptake of THO occurs within the first hour for the gills, within the first 30 minutes for the gut and within the first 10 minutes for the muscle tissue.

SPECIES DIFFERENCES

There are no differences in the percent saturation values of the gill tissues among the three species of decapods studied at an acclimation salinity of 100% sea water in the summer season. In 100%

sea water, during the summer, the gut of L. emarginata has percent saturation values that are lower than those of the other two species. The significance of this is not possible to assess at this time. In the winter, however, the permeability of the gills and gut of L. emarginata is higher than the permeability of the gills and gut of C. sapidus. The permeability of the tissues of both species drop in the winter, and the greatest differences in percent saturation values between the summer and the winter occurs in C. sapidus. The differences between the species appear to be correlated to the seasonal reduction in response of the two species.

At 40% sea water C. sapidus and C. irroratus gill and gut also have the same permeability to water. However, the osmolality values show that after acclimation to 40% sea water, C. sapidus is hyperosmoregulating to a greater extent than C. irroratus. These data suggest that the total osmoregulatory ability of a given species does not correlate solely with the water permeability of that species. This indicates that there are other factors involved in the response to an osmotic stress, such as active transport of salts and shifts of amino acids. (Croghan, 1958; Copeland, 1964; Mantel, 1967; Schoffeniels and Gilles, 1970).

Reduction in water permeability of the epithelial tissues upon acclimation appears to be a generalized pattern that is part of the total osmotic response of C. irroratus and C. sapidus to a change in salinity. This mechanism is of obvious advantage to an animal that inhabits an estuarine environment because such a reduction in input of water would limit the work necessary to balance the osmotic influx of water.

The response of the muscle tissue of the three species of decapods to an osmotic stress of 10‰ also appears to be a generalized pattern. The increase in THO uptake by the stressed muscle tissue corresponds to the behavior of stressed individual muscle fibers (Lang and Gainer, 1969). The changes in volume observed would be followed by the amino acid shifts that are necessary for intracellular isosmotic regulation.

Seasonality also effects the THO influx across the gill and gut tissues of C. sapidus and L. emarginata. The THO influx is reduced in the winter and increased in the summer season. These changes in permeability may be related to the general activity and the neuroendocrine state of the animals in the different seasons.

NEUROENDOCRINE EFFECTS

Work by Scudamore (1947), Kamemoto and Tullis (1972), Tullis and Kamemoto (1974), Mantel (1968), and Heit and Fingerman (1975) and others has established the presence of central nervous system (CNS) factors which affect the salt and water balance of decapod crustaceans. The results of this study show that CNS factors from the TGM and the ES decrease the water permeability of isolated gill and gut tissues of an osmoregulating and an osmoconforming decapod. The ES and TGM of crustaceans have been shown to contain cells responsible for neurosecretory activity (Bliss and Welch, 1952; Bliss et. al., 1954; Bliss, 1951; Passano, 1951). The results of this study suggest that more neurohormones that affect the reduction in water permeability are produced or stored in the TGM than in the ES, since the greatest

reduction in water permeability occurs when tissues are treated with TGM extracts (Table 5E). The gill tissue also appears to be more sensitive to the neurohormonal extracts than the gut (Table 5E). Many workers such as Maddrell (1962) have found that insect gut is particularly sensitive to neurohormones. Mantel (1968) has shown that isolated guts of G. lateralis respond to extracts made from the TGM of the same species. Mantel did not examine the permeability of the isolated gills of this species. However, the gills of the land crab are in a branchial chamber that is filled with air, not water; therefore, the gut of this species might be expected to play a more important role in hydromineral balance, as it does in insects. In fully aquatic crabs, the role of the gills as a major site of salt and water balance is well documented (Crogan, 1958; Copeland, 1964; Mantel, 1967). Hannen and Evans (1973) have calculated that the gills of U. pugilator are responsible for 86% of the THO influx. Therefore, it is understandable that the gill tissue of the crabs tested exhibit greater changes in THO influxes than the gut tissue when treated with neuroendocrine extracts.

L. EMARGINATA

The permeability of the gill tissue of L. emarginata was reduced significantly by extracts of the TGM and ES made from L. emarginata and C. sapidus. The permeability of the gut was reduced significantly by TGM extracts from 4 L. emarginata (Table 5E). It is interesting that the neurohormonal extracts necessary to reduce the permeability

of the epithelial tissues are present in the tissues of a crab that inhabits a fully marine environment and that is reported to be an osmoconformer (Gilles, 1970). Scudamore (1947); Bliss et. al., (1966); Mantel (1968); Carlisle (1955) and many others have demonstrated neuroendocrine regulation of ecdysial water balance in many species of crustaceans. Thus, there is a possibility that neuroendocrine factors that affect the movement of water at the time of ecdysis are the same as or similar to the factors that affect the water permeability throughout the molt cycle. Therefore, it is conceivable that an osmoconforming crab, such as L. emarginata would, throughout its molt cycle possess some of the neurohormones that control water transport in the stages surrounding ecdysis. L. emarginata is also known to undergo intracellular isosmotic regulation when exposed to a hyposmotic medium (Gilles, 1970). This implies that there are amino acid shifts in the tissues. Tucker and Costlow (1975) suggest that ES factors affect the free amino acid changes in the larvae of C. sapidus, therefore, it is also possible that the neurohormonal factors present in L. emarginata affect the intracellular isosmotic regulation.

The largest decrease in THO uptake occurs when the gills of L. emarginata are treated with extracts from the TGM of 2 C. sapidus acclimated to 40% sea water. This is to be expected, since an osmoregulating crab in a reduced salinity has a low permeability to water (Smith, 1970; Capen, 1972) and according to my results seems to have a greater amount of neurohormones present in its neurosecretory system. The results are different when the permeability of the gut tissue is examined. The only significant drop in the THO influx occurs

when the gut tissue of L. emarginata is treated with TGM extracts from 4 L. emarginata. The THO influx is reduced when extracts from C. sapidus are added but not significantly (Table 5C). The gut tissue appears to be more sensitive to extracts from the same species. However, if the dose of extracts from C. sapidus was increased, a greater reduction in permeability might be effected.

The present results indicate that the effects of the extracts are not species specific, since the extracts from C. sapidus affect the THO influx of the tissues of L. emarginata. This agrees with the results of Ramamurthi and Scheer (1967) who have shown that extracts from the prawn P. jordani affected the na^{+} efflux of H. nudius. Tullis and Kamemoto (1974) also found that extracts from T. crenata lower the permeability of the crayfish P. claricci. In a later paper, however, Kamemoto states that CNS extracts from the fresh water crayfish did not have the same effect on the water permeability of marine crabs. L. emarginata is a marine crab and C. sapidus is more of an estuarine animal. However, these differences in habitat and osmoregulatory ability do not seem to change the action of the neurohormones on the tissues.

C. SAPIDUS

The THO influxes across the gill and gut tissues of C. sapidus acclimated to 40% sea water are not reduced significantly when treated with extracts made from 4 C. sapidus acclimated to 40% sea water. The THO influx is reduced somewhat when the tissues are treated either with

TGM or ES extracts, however, the THO influx of these tissues is very low (mean % saturation = 6.6) without extracts. Therefore, a further reduction in permeability may be impossible. The mechanism of neurohormonal action is unknown. However, these experiments suggest that whatever mechanism is responsible could reach a saturation point beyond which an excess of neurohormone has no effect.

The permeability of the gill tissues of C. sapidus acclimated to 100% sea water is reduced significantly when treated with TGM extracts from 4 C. sapidus acclimated to 100% sea water and from 2 C. sapidus acclimated to 40% sea water. The greatest change in percent saturation occurs on treatment with extracts from 4 C. sapidus acclimated to 100% sea water. Preliminary experiments examined the permeability of the gill and gut tissues of C. sapidus treated with extracts made from 2 C. sapidus acclimated to 100% sea water. Under these experimental conditions there were no differences between the tissues treated with extracts and the water controls. Therefore, the dosage was increased by using extracts from 4 instead of 2 animals. The increased dosage of TGM extract resulted in a decrease in the THO influx across the gill tissue. The ES extract did not result in a decrease. These data suggest that some of the neuroendocrine factors are present even when this species is osmoconforming in 100% sea water. Treatment of the gill tissue with extracts made from 2 C. sapidus acclimated to 40% sea water decreases the THO influx. The TGM extracts reduce the permeability significantly; the ES extracts reduce the permeability, but not significantly (Table 5C). These results indicate that more, or stronger, neurohormonal factors are present in animals that are

osmoregulating, since extracts from 2 animals produce a significant effect. If the dosage was increased to extracts made from 4 C. sapidus acclimated to 40% sea water, a greater change in permeability probably would have been effected. The permeability of the gut tissue of C. sapidus acclimated to 100% sea water was not reduced significantly by treatment with any of the extracts. This agrees with the general pattern of reduced sensitivity of the gut tissues of the two species to the neurohormonal extracts. The largest reduction in THO influx, however, occurs when the gut tissue is treated with extracts from the TGM of 4 C. sapidus acclimated to 100% sea water ($p < .1$). This is the same treatment that causes the greatest reduction in the THO influx of the gill tissue. Increasing the dosage of extracts from animals acclimated to 40% sea water from 2 to 4 most probably would reduce the THO influx to a statistically significant level.

Kamemoto (1976) in his review paper indicates some of the inconsistencies in the results obtained when extracts of CNS tissues are injected into whole animals. In some instances, extracts from the same neuroendocrine tissue appear to have the opposite effect. Kamemoto and Tullis (1974) and Tullis (1975) have separated extracts from the TGM and the brain of the euryhaline decapod T. crenata. They report the presence of two fractions, a water-soluble fraction that decreases the THO influx of the whole animal and the gills and an acetone-soluble fraction that increases the THO influx. Crude extracts probably contain multiple hormonal factors; this has been shown in the chromatophore controlling hormones (Rao et. al., 1967; Fingerman

et. al., 1971). Bliss et. al., (1966) have also suggested the presence of a diuretic and antidiuretic hormone in G. lateralis.

The CNS tissues in the present study were extracted in water. The results of the THO influxes of the isolated tissues treated with these extracts agree with the results that Kamemoto and Tullis observed on whole animals; that is, there was a decrease in water influx upon treatment with CNS extracts. However, Mantel (1968) shows conclusively that TGM extracts increase the THO influx across the isolated gut of G. lateralis. She also states that boiling the extract destroys its activity. All extracts in the present experiment were boiled for 10 minutes before being used; this could account for some of the differences in the observed effect of the extract. The concept of multiple hormonal factors has been discussed above. Perhaps unboiled crude extract contains more of the factors that tend to increase the water permeability. In addition, Mantel's experiments were carried out on G. lateralis, the true land crab. This species is adapted to a terrestrial existence, since the only time that it must return to water is to shed its eggs. Therefore, this species is adapted to live in an environment that imposes different physiological constraints than does an aquatic environment. It is likely that the neurohormonal system that is present in any given species of decapod has evolved to cope with the physiological problems of that species. Therefore, it is conceivable that extracts from the CNS tissues may have the opposite effects on the same tissues from different species of crabs which are adapted to live in radically different environments. Kamemoto (1974) and Kamemoto and Tullis (1972) have partially isolated a factor that

when injected into crayfish, causes an increase in the concentration of salts in the hemolymph and an increase in the Na influx. This factor is found in the CNS of fresh water decapods or in terrestrial decapods associated with fresh water and is not found in marine decapods. Extensive work must be done in order to obtain purified CNS extracts that can be tested in whole animal and on isolated tissue in order to determine the effects extracts have on the same tissues of different species.

The reduction of water permeability of the gill and gut tissues on treatment with neuroendocrine extracts appears to be consistent for the two species of crabs that were studied. Both the osmoconforming and the osmoregulating species possess hormonal factors concentrated in their neuroendocrine tissues when they are acclimated to 100% sea water or to 40% sea water. However, the crabs that were acclimated to 40% sea water appeared to have a greater concentration of the neuroendocrine factors since these extracts generally produce the greatest change in permeability to water. The neuroendocrine factors were not species specific; the extracts from C. sapidus caused changes in the water permeability of the tissues of L. emarginata.

SUMMARY

1. Cancer irroratus, Callinectes sapidus, and Libinia emarginata are osmoconforming when they are acclimated to 100% sea water.
2. C. sapidus and C. irroratus are hyperosmoregulating when they are acclimated to 40% sea water. C. sapidus is the stronger regulator.
3. C. sapidus and C. irroratus can reduce the water permeability of their epithelial tissues on acclimation to a lower salinity.
4. The muscle tissue of C. sapidus and C. irroratus exhibit no generalized change in permeability on acclimation to a lower salinity.
5. The gill and gut tissues of all three species of decapods do not exhibit any change in permeability to water in response to a 10% osmotic stress.
6. The THO uptake of the muscle tissue of all three species of decapods subjected to a 10% osmotic stress is significantly higher than the THO uptake of muscle tissue not subjected to an osmotic stress. This is probably related to changes in cell volume associated with intracellular isosmotic regulation.
7. Seasonality affects the influx of THO across the gill and gut tissues of L. emarginata and C. sapidus. The THO influx is reduced in the winter, this corresponds to the reduction in general activity of both species.
8. Neuroendocrine extracts from the TGM and ES reduce the permeability to water of the gill and gut tissues of both species.

9. Both L. emarginata and C. sapidus acclimated to 100% sea water have hormonal factors present in their neuroendocrine tissues.
10. C. sapidus acclimated to 40% sea water appeared to have more or stronger hormonal factors in its TGM since extracts of TGM generally caused the greatest reduction in permeability to water.
11. The neuroendocrine factors were not species specific in their action.
12. The gill tissue exhibited a greater response to the neurohormonal factors than the gut tissue.

BIBLIOGRAPHY

Abramowitz, R., and Abramowitz, A., 1940.
"Molting, growth, and survival after eyestalk removal in UCA pugliator."
Biol. Bull. 78: 179-188.

Bliss, D.E., 1951.
"Metabolic effects of sinus gland or eyestalk removal in the land crab G. lateralis."
Anat. Rec. III: 502-503.

Bliss, D.E., and Welsh, J.H., 1952.
"The neurosecretory system of the bracttyuran crustacea."
Biol. Bull., 103: 157-169.

Bliss, D.E., Durand, J.B., and Welsh, J.H., 1954.
"Neurosecretory systems in decapod crustacea."
Z. Zellforsch. 30: 520-536.

Bliss, D.E., Wang, S.M., and Martinez, E.A., 1966.
"Water balance in the land crab, Gecarcinus lateralis, during the intermolt cycle."
Am. Zoologist. 6: 197-212.

Cantelmo, A.C., Cantelmo, F.R., and Langsam, D.M., 1975.
"Osmoregulatory ability of the rock crab, Cancer irroratus, under osmotic stress."
Comp. Biochem. Physiol. 51A: 537-542.

Capen, R.L., 1972.
"Studies of water uptake in the euryhaline crab, Rhithropanopeus harrisii."
J. Exp. Zool. 182: 307-320.

Carlisle, D. B., 1955.
"On the hormonal control of water balance in Carcinus."
Pubbl. Staz. Zool. Napoli. 27: 227-231.

Copeland, D.E., 1964.
"Salt absorbing cells in the gills of crabs Callinectes and Carcinus."
Biol. Bull. 127: 367 (abstr.).

Cornell, J.C., 1974.
"A reduction in water exchange rates in an osmoconforming crab."
Am. Zool. 14: 4: 1259 (abstr.).

Croghan, P. C., 1958.
"The osmotic and ionic regulation of Artemia salina."
L. J. Exptl. Biol. 35: 219-233.

Dall, W., 1965.
"Studies on the physiology of a shrimp, Metapenaeus sp. (crustacea: decapoda: penaeidae) III. Composition and structure of the integument."
Australian J. Marine Freshwater Res. 16: 13-23.

Dall, W., 1967.
"The funtional anatomy of the digestive tract of a shrimp Metapenaeus bennettae Racek and Dall (crustacea: decapoda: penaeidae)."
Australian J. Zool. 15: 699-714.

Dandifosse, G., 1966.
"Absorption d'eau au moment de la mue chez un crustacé décapode: Maia squinado."
Herbst. Arch. Intern. Physiol. Biochem. 74: 329-331.

Drach, P., 1939.
"Mue et cycle d'intermue chez les crustacés décapodes."
Annuls. Inst. Oceanogr., Paris, (N.S.) 19: 103-391.

Fingerman, M., Bartell, C.K., and Krasnow, R.A., 1971.
"Comparison of chromatophorotropins from the horseshoe crab Limulus polyphemus, and the fiddlercrab UCA pugilator."
Biol. Bull. 140: 376-388.

Gerard, J.F., 1975.
"Volume regulation and alanine transport response of isolated axons of Callinectes sapidus rathbun to hypo-osmotic conditions."
Comp. Biochem. Physiol. 51A: 225-229.

Gerard, J.F., and Gilles, R., 1972.
"Modification of the amino-acid efflux during the osmotic adjustment of isolated axons of Callinectes sapidus."
Experientia 8: 863-864.

Gifford, C.A., 1962.
"Some aspects of osmotic and ionic regulation in the blue crab, Callinectes sapidus, and the ghost crab, Ocypode albicans."
Publs. Inst. Marine Sci., Texas, 8: 97-125.

Gilles, R., 1970.
"Osmoregulation in the stenohaline crab Libinia emarginata (Leech)."
Arch. Inter. Physiol. et. Biochem. 78: 91-99.

Gilles, R., 1972.
"Osmotic behavior of isolated axons of a euryhaline and a stenohaline crustacean."
Experientia 11: 29, 1354-1355.

Gross, W.J., 1957.
"An analysis of response to osmotic stress in selected decapod crustacea."
Biol. Bull. 112: 43-64.

Gross, W.J., 1964.
"Trends in water and salt regulation among aquatic and amphibious crabs."
Biol. Bull. 127: 447-466.

Haberfield, E.C., Hass, L.W., and Hammen, C.S., 1975.
"Early ammonia release by a polychaete Nereis virens and a crab Carcinus maenas in diluted sea water."
Comp. Biochem. Physiol. 52A: 501-503.

Hannen, J.V., and Evans, D.H., 1973.
"Water permeability in some euryhaline decapods and Limulus."
Comp. Biochem. Physiol. 44A: 1199-1213.

Heeg, J. and Cannone, A.J., 1966.
"Osmoregulation by means of a hitherto unsuspected osmoregulatory organ of two grapsid crabs."
Zool. Africana 2: 127-129.

Heit, M., and Fingerman, M., 1975.
"The role of an eyestalk hormone in the regulation of the sodium concentration of the blood of the fiddler crab, UCA pugilator."
Comp. Biochem. Physiol. 50A: 277-280.

Hinsch, G.W., 1972.
"Some factors controlling reproduction in the spider crab, Libinia emarginata."
Biol. Bull 143: 2, 358-366.

Kamemoto, F.I., 1974.
"Neuroendocrine adaptation in salt regulation in freshwater and terrestrial crabs."
Am. Zool. 14: 1243.

Kamemoto, F.I., 1976.
"Neuroendocrinology of osmoregulation in decapod crustacea."
Amer. Zool. 16: 141-150.

Kamemoto, F.I., Kato, K.N., and Tucker, L.E., 1966.
"Neurosecretion and salt and water balance in the Annelida and Crustacea."
Am. Zoologist. 6: 213-219.

Kamemoto, F.I. and Tullis, R.E., 1972.
"Hydromineral regulation in decapod crustacea."
Gen. Comp. Endocrinology, Suppl. 3: 299-307.

Kinne, O., 1971.
Marine Ecology, Vol. I, part 2.
Wiley-Interscience, New York.

Kleinholz, L.H., 1976.
"Crustacean neurosecretory hormones and physiological specificity."
Amer. Zool. 16: 151-166.

Krogh, A., 1938.
"The absorption of ions in some freshwater animals."
Z. Vergl. Physiol. 25: 345-350.

Lang, M.A., and Gainer, H., 1969.
"Volume control by muscle fibers of the blue crab."
J. Gen. Physiol. 53: #3, 323-341.

Lockwood, A.P.M., 1965.
"The relative losses of sodium in the urine and across the body surface in the amphipod Gammarus deubeni."
J. Exp. Biol. 42: 59-69.

Lockwood, A.P.M., 1968.
"Aspects of the physiology of crustacea."
Oliver and Boyd, London: 1-328.

Lockwood, A.P.M., and Andrews, W.R.H., 1969.
"Active transport and sodium fluxes at molt in the amphipod Gammarus deubeni."
J. Exp. Biol. 51: 591-605.

Lockwood, A.P.M., and Inman, C.B.E., 1973.
"Changes in the apparent permeability to water at molt in the amphipod, Gammarus duebeni and in the isopod Idota linearis."
Comp. Biochem. Physiol. 44: 3A 943.

Lynch, M.P., Webb, K.L., and VanEngel, W.A., 1973.
"Variations in serum constituents of the blue crab, Callinectes sapidus: chloride and osmotic concentration."
Comp. Biochem. Physiol. 44A: 719-734.

Maddrell, S.H.P., 1962.
"A diuretic hormone in Rhodnius prolixus."
Nature, 194: 605-606.

Mantel, L.H., 1965.
"A symmetry potentials, metabolism, and ion fluxes in the gills of the blue crab, Callinectes sapidus."
Ph.D. Thesis, University of Illinois, Urbana, Illinois.

Mantel, L.H., 1967.
"Asymmetry potentials, metabolism and sodium fluxes in gills of the blue crab, Callinectes sapidus."
Comp. Biochem. Physiol. 20: 743-753.

Mantel, L.H., 1968.
"The foregut of Gecarcinus lateralis as an organ of salt and water balance."
Am. Zoologist, 8: 433-442.

Mantel, L.H., Bliss, D.E., Sheehan, S.W., and Martinez, E.A., 1975.
"Physiology of hemolymph, gut fluid, and hepatopancreas of the land crab Gecarcinus lateralis (Fréminuille) in various neuroendocrine states."
Comp. Biochem. Physiol. 51A: 663-671.

Mantel, L.H., and Olson, J.R., 1976.
"Studies on the Na^+ K^+ activated ATPase of crab gills."
Amer. Zool. 16: 223, 269, (Abstr.).

Nagel, H., 1934.
"Die aufgaben der exkretionsorganc und der kieman bei de osmoregulation von Carcinus maenas."
Z. Vergl. Phys. 21: 468-491.

Passano, L.M., 1951.
"The X organ-sinus gland neurosecretory system in crabs."
Anat. Rec. III: 502.

Potts, W.T.W., and Parry, G., 1964.
Osmotic and Ionic Regulation In Animals, 423.
Macmillan Company, New York.

Prosser, C.L., and Brown Jr., F., 1961.
Comparative Animal Physiology.
Saunders, Philadelphia.

Quinn, D.J., and Lane, C.E., 1966.
"Ionic regulation and Na^+ K^+ stimulated ATPase activity in the land crab, Cardisoma guanhumi."
Comp. Biochem. Physiol. 19: 533-543.

Rammamurthi, R., and Scheer, B.T., 1967.
"A factor influencing sodium regulation in crustaceans."
Life Sci. 6: 2171-2175.

Rao, K.R., Fingerman, M., and Bartell, C.K., 1967.
"Physiology of the white chromatophores in the fiddler crab, UCA pugilator."
Biol. Bull. 133: 606-617.

Rudy, P.P., 1967.
"Water permeability in selected decapod Crustacea."
Comp. Biochem. Physiol. 22: 581-589.

Schoffeniels, E. and Gilles, R., 1970.
Osmoregulation in Aquatic Arthropods. In M. Florkin and B.T. Scheer (eds).
Chemical Zoology, Vol. V., 255-286.
Academic Press, New York.

Scudamore, H.H., 1947.
"The influence of the sinus glands upon molting and associated changes in the crayfish."
Physiol. Zool. 20: 187:208.

Seibers, D., and Lucu, C., 1973.
"Mechanisms of intracellular isosmotic regulation: extracellular space of the shore crab Carcinus maenas in relation to environmental salinity."
Helgoländer Wiss. Meeresunters 25: 199-205.

Skinner, D.M., Marsh, D.J., and Cook, J.S., 1965.
"Physiological salt solution for the land crab, Gecarcinus lateralis."
Biol. Bull. 129: 355-365.

Smith, R.I., 1967.
"Osmotic regulation and adaptive reduction of water permeability in the brackish water crab, Rhithropanopeus harrisii (Brachyura Xanthidae)."
Biol. Bull. 133, 643-658.

Smith, R.I., 1970.
"The apparent water permeability of Carcinus meanas (Crustacea, Brachyura, Portunidae) as a function of salinity."
Biol. Bull. 139: 351-362.

Smith, R.I., and Rudy, P.P., 1972.
"Water exchange in the crab H. nudus measured by the use of Duterium and Tritium oxidesactracers."
Biol. Bull. 143: 234-246.

Sokal, R.R., and Rohlf, F.J., 1973.
Introduction to Biostatistics.
W.H. Freeman and Company.
San Francisco.

Tagatz, M.E., 1971.
"Osmoregulatory ability of blue crabs in different temperature-salinity combinations."
Chesapeake Sci. 12: 14-17.

Tan, Eng-Chow, and VanEngel, W.A., 1966.
"Osmoregulation in the adult blue crab, Callinectes sapidus (Rathbun)."
Chesapeake Sci. 7: 30-35.

Towle, D.W., and Taylor, D.D., 1976.
"Effect of NH_4^+ and K^+ on Na^+ transport ATPase activity of blue crab gill."
Amer. Zool. 16: 224, #270, (Abstr.).

Tucker, R.K., and Costlow Jr., J.D., 1975.
"Free amino acid changes in normal and eyestalkless megalopa larvae of the blue crab, Callinectes sapidus during the course of the molt cycle."
Comp. Biochem. Physiol. 51A: 75-78.

Tullis, R.E., 1975.
"CNS factors affecting water and sodium efflux from decapod crustaceans."
Amer. Zool. 15: 786.

Tullis, R.E., and Kamemoto, F.I., 1974.
"Separation and biological effects of CNS factors affecting water balance in the decapod crustacean Thalamita crenata."
Gen. and Comp. Endocrinol 23: 19-28.

Warner, W.W., 1976.
Beautiful Swimmers.
Little, Brown and Company.
Boston - Toronto.

Weber, R.E. and Spaargaren, D.H., 1970.
"On the influence of temperature on the osmoregulation of Crangon crangon and its significance under estuarine conditions."
Neth. J. Sea. Res. 5: 108-120.